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Soluble manganese as a factor affecting the growth of various legumes in culture solutions and in acid soils

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SOLUBLE MANGANESE AS A FACTOR AFFECTING THE GROWTH OF VARIOUS LEGUMES IN CULTURE SOLUTIONS AND IN ACID SOILS

by

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INTRODUCTION

One of the oldest and most widely accepted agronomic concepts is that plant growth on strongly acid soils is greatly benefited by applications of lime. Despite the common recognition of the beneficial effects from liming acid soils, there is little agreement as to the relative importance of the factors responsible for these beneficial effects on different soils and with various crop plants. Numerous investigators have attributed the relatively poor growth of plants on acid soils to several factors, namely: direct acidity effects, low calcium status, low calcium or base saturation, inhibition of beneficial microbiological activities, fixation of phosphorus in unavailable forms, and toxicity from increased solubility of iron, aluminum and manganese.

Legumes, as a whole, are considered to be especially benefited by liming. However, it is generally recognized that various legumes differ greatly in their ability to tolerate acid soil conditions. This belief is so prevalent that legumes are commonly classified, agronomically, according to their sensitivity or tolerance to soil acidity. Results from preliminary investigations, studying the growth of various legumes under acid soil conditions, indicated that soluble manganese might be an important factor affecting the growth of legumes on certain acid soils.

The purpose of this investigation was to evaluate the role of soluble manganese as a possible injurious factor in the growth of legumes under acid soil conditions. Supplementing this study, it appeared desirable to compare the relative tolerance of several legumes to soluble manganese as a possible explanation for their differential growth on acid soils, and to determine the effect of varying concentrations of other nutritive elements upon the tolerance of legumes to soluble manganese.

REVIEW OF LITERATURE

Possible Importance of Soluble Manganese as a Factor Affecting Plant Growth on Acid Soils

The manganese toxicity concept is not a recent development. Ewell (10), in 1902, noted that legumes grew very poorly on acid soil which contained considerable amounts of water-soluble manganese. Water extracts of this soil contained approximately twice as much manganese as calcium. Ewell believed that this high proportion of manganese in the soluble salts of the soil contributed largely to its sterility.

Nagaoka (37), in 1906, applied manganese to rice paddy soils at a rate of 50 pounds per acre. For two years an increase in the rice yield was obtained but then a decrease in yield resulted where the manganese was supplied as the sulfate or chloride while applications of manganese carbonate apparently had no effect. The reduction in yield in the case of manganese chloride and sulfate was believed to be due to the increased acidity of these plots as compared to the check or manganese carbonate plots. However, it is more likely that the additional soluble manganese may have been the injurious factor responsible for the poor growth rather than the soil acidity.

Skinner and Reid (51), in 1916, and McHargue (36), in 1923, working along somewhat similar lines, found that additions of soluble manganese to acid soils decreased yields of such crops as wheat, rye, corn, cowpeas, potatoes, radish, and soybeans. However, when these soils were limed to approximate neutrality, manganese was found to be beneficial to all the above crops except potatoes. From his work McHargue concluded that soluble manganese may be one of the causes of toxicity in such acid soils.

Kelley (25, 26, 27, 28) investigated the poor growth of pineapples on "black soils" of Hawaii and concluded that the relatively high manganese concentration of these soils was the injurious factor responsible for the poor growth. The "black soils" were found to be less acid than the normal "red soils", and lime or fertilizer treatments did not effectively overcome the injury to pineapples.

Guthrie and Cohen (16) found that soil on which grass and barley failed to grow contained a much higher concentration of manganese than nearby soil supporting good crop growth. Both soils were neutral in reaction. In view of the present knowledge of the insolubility of manganese at a pH value approaching neutrality there is some doubt that toxicity in this case was due to manganese.

Jacobson and Swanback (22) reported that manganese toxicity was responsible for the stunted and chlorotic growth of tobacco on certain acid Connecticut soils. They based their conclusions on the relatively high "active" manganese in the soil, the extremely high manganese content of the plant, and the identical toxicity symptoms obtained when tobacco was grown in dilute manganese solutions. Bortner (5), using essentially the same method in studying the problem as Jacobson and Swanback, also concluded that poor growth of Turkish tobacco on acid Kentucky soils was due to soluble manganese.

Parberry (40) observed that beans showing severe toxicity symptoms contained more than twice the manganese content of normal plants. Application of lime to the soil on which the abnormal plants were growing resulted in normal plant growth and greatly reduced the manganese content of the plants.

Hale and Heintze (18) found that potatoes, sugar beets, lettuce and kale exhibiting toxicity symptoms contained much higher concentrations of manganese than normal plants growing in the immediate vicinity of the abnormal plants. Analyses of the soils on which the poor plants were growing showed that they contained more exchangeable manganese than the adjacent soil on which plant growth was normal.

Wallace, Hewitt and Nicholas (57), in examining crop failures, studied visual symptoms on plants in the field and in sand cultures and concluded that toxicity symptoms in the field were due to excess manganese rather than to a lack of calcium.

Factors Affecting the Concentration of Soluble Manganese in Soils

Only a few studies have been reported of supposed manganese toxicity occurring on other than rather strongly acid soils (16, 24). This fact is readily explained, as the soluble divalent form of manganese is oxidized to an insoluble form under aerobic conditions at pH values approaching neutrality. Therefore, any factor increasing the acidity of a moderately acid soil might be expected to increase the concentration of soluble manganese in that soil.

Numerous investigations have been reported where applications of acid-forming fertilizers over a period of years have resulted in greatly decreased crop yields as compared to the yields obtained with neutral or basic fertilizers. The differential effect of the fertilizers might be explained, in some instances at least, by the increased concentration of soluble manganese in the soils receiving the acid-forming fertilizers. Funchess (12) reported that applications of acid-forming nitrogenous fertilizers to Alabama soils over a period of years resulted in greatly reduced plant growth. Concentrations of water-soluble manganese as high as 84 p.p.m. (soil basis) were found in water extracts of soils receiving applications of ammonium sulfate, dried blood, or cotton-seed meal whereas no soluble manganese was found in the untreated or limed plots where plant growth was normal. Lindsey (31) determined the water-soluble manganese, aluminum, and iron in soil from fertilizer plots which had received applications of ammonium sulfate annually for 32 years and found approximately 65 p.p.m. manganese, a smaller quantity of aluminum, and only a trace of iron. Ames and Boltz (1) found that ammonium sulfate, sulfur, and dried blood all increased the water-soluble manganese in soils.

Fried and Peech (11) found that two tons of calcium sulfate per acre increased soil acidity from pH 4.80 to 4.65 and the manganese concentration of the soil solution from 3 to 40 p.p.m. They credited the increase in soluble manganese to the concurrent increase in soil acidity and replacement of exchangeable manganese by calcium.

Smith (52) applied heavy applications of potassium, either as the chloride or sulfate, to acid soils and obtained marked increases in the water-soluble manganese content of the soils. The soil highest in water-soluble manganese showed the least nitrifying efficiency, the smallest growth of wheat in pot cultures, and the poorest growth of wheat rootlets in soil extract cultures.

Piper (44), Godden and Grimmett (15), and Robinson (47) have reported increased solubility of soil manganese as a result of poor soil drainage. McCool (34) observed that decaying organic matter, high moisture content, and steam sterilization all increased the water-soluble manganese concentration of several soils. He also found that plants growing in full sunlight absorbed more manganese than plants growing in shade. This may be due in part at least, to the increased available soil manganese at the higher temperature.

Mann (32) studied the effects of applications of magnesium and calcium carbonates on two Coastal Plain soils and found that either was very effective in reducing the water-soluble manganese in these soils. Other investigators (5, 11, 22) have reported similar results with calcium carbonate on different soils. Sherman and Fujimoto (50) found that a soil mulch lowered the soil temperature and reduced exchangeable manganese almost as effectively as applications of calcium hydroxide.

Applications of phosphate fertilizer have been observed to be beneficial to plants growing on soils containing toxic amounts of soluble manganese (5, 26) but no data have been reported to show that the beneficial effect was due to a decrease in the solubility of the soil manganese.

Jacobson and Swanback (22) found that the "active" manganese concentration of one soil was decreased somewhat by phosphorus but was not affected with another soil. The manganese content of the plants growing on the phosphorus treated soils was higher in both cases than that found in plants from the untreated soils.

According to Britton (7) divalent manganese is not precipitated as phosphate except at a reaction of pH 5.76 or higher. At this moderate acidity there is not likely to be toxic concentrations of soluble manganese present in soils with the possible exception of those of Hawaii. Therefore such beneficial effects as reported by Pierre and Stuart (43) in the reduction of aluminum toxicity by heavy applications of phosphate would not appear probable in the case of manganese toxicity.

Relative Tolerance of Plant Species to Soluble Manganese

Although numerous instances have been reported of manganese toxicity to various plant species, little is known as to the comparative tolerance of various plants to soluble manganese. This is especially true in regard to legumes. Kelley (27), in a thorough study of plant growth on the high-manganese soils of Hawaii, found that various plants differed greatly in their ability to grow on these soils. Among the non-legumes grown, pineapples and corn were definitely injured and showed definite toxicity symptoms while cotton, potatoes, cabbage and turnips were not affected. Legumes exhibited a wide range in the degree of tolerance to manganese toxicity; crotalaria was not affected; peanuts and kidney beans were moderately sensitive; and cowpeas were very sensitive.

Johnson (23), also working with Hawaiian soils, reported a similar classification of crops; peanuts, cowpeas, pigeon peas, corn, and sugar cane grew poorly on the high manganese soils while crotalaria, oats, wheat and tobacco were not affected. The only criticism of these investigations is that other factors such as phosphate deficiency may have affected plant growth on these soils and been responsible for the differential growth of the various plants. The total amounts of manganese in these soils were given but no data were supplied as to the actual concentration of soluble manganese present in the soil.

More recently, Olsen (39) determined the toxicity of manganese to various plant species in culture solutions and found that some species (Lemna polyrrhiza and Senecio silvaticus) were injured by concentrations of manganese as low as 0.5 p.p.m. while corn was injured only when the manganese concentration was raised to 62.5 p.p.m. It is of interest to note that the minimum concentration of manganese causing injury, 0.5 p.p.m., was of the same magnitude as that reported by Ligon and Pierre (30) for aluminum toxicity.

Jacobson and Swanback (21, 22) grew tobacco in nutrient solutions containing concentrations of manganese sulfate varying from 0 to 12 p.p.m. Toxicity was found to occur at concentrations below 1.0 p.p.m. manganese sulfate. Bortner (5) also reported that tobacco was injured by a concentration of 15 p.p.m. manganese when grown in culture solutions. Brenchley (6) found that approximately 3.5 p.p.m. manganese in nutrient solutions retarded growth of barley. Peas were more sensitive to manganese than barley. Gilbert and Pember (14) obtained a 25 per cent decrease in yield of red clover when the concentration of manganese in the nutrient solution was raised from 1 to 10 p.p.m.

Hopkins, et al.(20) grew tomatoes, beans and pineapples in culture solutions and found that they differed significantly in their tolerance to soluble manganese. Pineapples were most tolerant, beans intermediate, and tomatoes most sensitive to manganese toxicity.

The Effect of the Concentration of Other Nutritive Elements on Manganese Toxicity

General knowledge of the absorption of nutritive ions by plant roots indicates that the amount of manganese absorbed by plants would be determined lafgely by the relative concentration of manganese available to the roots as compared to the total concentration of other ions. The work of McCool (33), Deatrick (9) and others supports this conclusion. They found that the concentration of manganese necessary for injury to plants was greater in nutrient solutions containing a high total concentration of salts than in distilled water or more dilute nutrient solutions. Hewitt and his associates (19, 57) have reported reduced toxicity from manganese by increasing the calcium concentration in culture solutions. Chapman (8) on the other hand found that manganese toxicity was increased by a high concentration of calcium.

Applications of soluble phosphates to soils containing high amounts of manganese were beneficial to plant growth according to Kelley (27) and Bortner (5). Bortner also obtained similar results with tobacco grown in culture solutions with and without phosphorus. McCool (34) and Jacobson and Swanback (22) reported no beneficial effect on manganese toxicity from applications of phosphates.

The importance of the iron and manganese balance in plants has been known for a considerable length of time. Brenchley (6) reported that additions of ferric chloride to culture solutions eliminated the chlorosis of wheat and oats resulting from manganese toxicity. Pugliese (45) grew cereals in culture solutions to which were added varying amounts of ferrous sulfate and manganese salts. He found that concentrations of manganese nitrate greater than 50 p.p.m. were detrimental without iron but were stimulatory if from 70 to 150 p.p.m. ferrous sulfate was added. He concluded that in the presence of iron the plants could tolerate very much larger concentrations of manganese.

Tottingham and Beck (55), Johnson (24) and Rippel (46) have reported similar beneficial effects from iron in reducing manganese toxicity. More recent work on the roles of iron and manganese in plant metabolism has been carried out by Somers and Shive (53, 54), working with soybeans. They concluded that the theoretical explanation of the iron and manganese relationship in plant metabolism is dependent upon two facts: 1) that the active functional iron is in the ferrous condition; 2) that the oxidation potential of manganese is higher than that of iron. Iron in the plant cells is reduced to the ferrous state unless this reaction is prevented by some counter-reactant. If such a reactant is not present in the cell then a small amount of ferrous iron may be exceedingly toxic and produce iron toxicity symptoms which they claimed are identical with those of manganese deficiency. On the other hand, if the proper amount of manganese is present in the plant a balance is obtained between the ferrous and ferric states of iron and normal growth results; whereas if an excess of manganese is present the iron will be oxidized and precipitated in an immobile form in the plant tissue, and the plants will show symptoms of manganese toxicity or iron deficiency. They stated that the optimum soluble iron-manganese ratio in the substrate and in the plant was between 1.5 and 2.5 and that any deviation from these limits results in iron or manganese toxicity.

Pearse (41) obtained optimum growth of gooseberries and strawberries in culture solutions with an iron-manganese ratio of approximately 2.5. Hopkins, et al. (20) found that an increase in the iron concentration of nutrient solutions reduced manganese toxicity symptoms and increased growth of beans. However, optimum growth was obtained at much higher iron-manganese ratios than 2 and in no case was iron toxicity evident. They concluded that the discrepancy between their results and those of Somers and Shive (54) was due to two factors; namely, the different forms of iron used, and the different light intensities prevalent during the two experiments.

In general, the majority of the investigations reported have more or less supported Somers and Shive's theory concerning the iron-manganese relationship in plants. However, Haas (17) found that excess concentrations of manganese brought about chlorosis of citrus even though iron was added to the culture solutions in large amounts.

THE MINIMUM CONCENTRATIONS OF MANGANESE NECESSARY FOR INJURY TO VARIOUS LEGUMES IN CULTURE SOLUTIONS

Introduction

There is little information as to the minimum concentrations of soluble manganese that cause injury to various legumes. Comparison of the results of different investigators as to the tolerance of various legumes to manganese toxicity would be misleading in view of the fact that no standard experimental procedure is followed, and variables other than the concentration of manganese would make any reliable conclusions difficult.

It is not clearly known why legumes differ so markedly in their ability to grow well under acid soil conditions. Theories have been advanced that differences in calcium requirement of legumes or tolerance to soluble aluminum are responsible for the differential growth.

The specific objectives of these experiments were as follows:

1) To determine the range of manganese concentrations in culture solutions at which injury to the growth of legumes is obtained.

2) To compare the relative tolerance of several legumes

to soluble manganese as a possible explanation of their differential growth on acid soils.

Experimental

General procedure

The five legumes used in the study were: lespedeza, soybeans, cowpeas, peanuts, and sweet clover. The seeds were planted in sand and the seedlings transferred to the culture solutions when approximately two inches in height. All experiments were conducted in a greenhouse which was whitewashed during the months of July, August and September to aid in controlling the temperature.

A modified Hartwell and Pember culture solution similar to that employed by Ligon and Pierre (30), except that it contained from 0.25 to 0.50 p.p.m. boron as boric acid, was used in all experiments. In order to provide sufficient manganese for normal plant growth, 0.1 p.p.m. manganese was also added to the standard culture solution.

Culture vessels were glazed, earthenware pots of 8-liter capacity, covered with painted, galvanized lids containing four holes sufficiently large to hold paraffined corks 2.5 inches in diameter. The plants were placed in holes bored in the cork and held in place by cotton padding. Manganese was supplied as manganese chloride. Distilled water was used throughout in preparing culture solutions. All cultures were aerated for two 15-minute periods each day during the growing period. Treatments were randomized in complete blocks and rerandomized at weekly intervals during the experiments. The pH values of the solutions were maintained at 4.6 and adjusted if necessary with either sulfuric acid or sodium hydroxide. Phosphorus determinations were made at frequent intervals but in only one case was the phosphorus concentration lowered more than 10 per cent or enough to necessitate addition of phosphate.

The treatments consisted of five different concentrations of manganese, varying from 0.1 to 10 p.p.m. except in the sweet clover experiment where the highest rate of manganese was omitted. One additional treatment was included in the soybean, cowpea, and sweet clover experiments to study the effect of maintaining a hydrogen-ion concentration of pH 6.0 on the growth of these legumes. Treatments were replicated three times in the lespedeza, peanut, and sweet clover experiments and twice in the other experiments. A general view of the apparatus used in all the culture solution experiments is given in Fig. 1.

Cultural procedure

Lespedeza experiment. Two strains of Korean lespedeza,

Fig. 1. General view of culture solution apparatus.

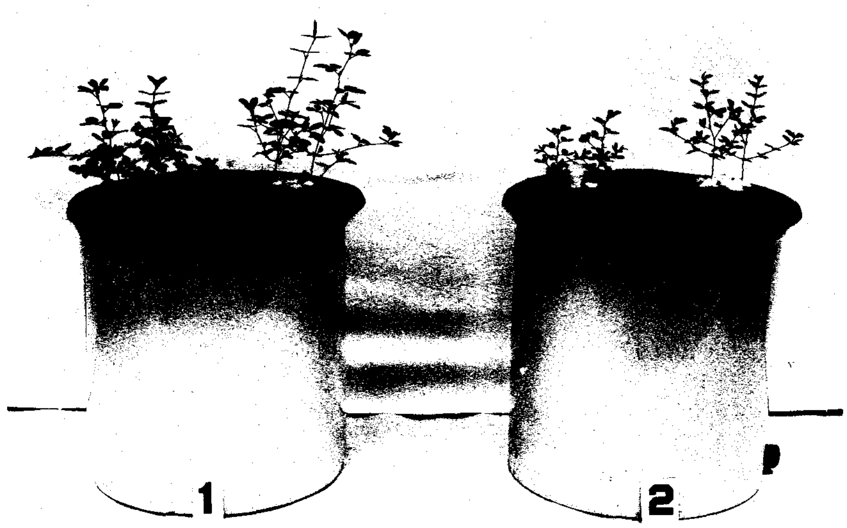


Fig. 2. Growth of lespedeza in culture solutions of different manganese concentration. Strain L29 at left and strain L6 at right in each container.
1. 0.1 ppm Mn   2. 10.0 ppm Mn

L6 and L39[1] were used in this experiment. Some evidence had been obtained previously that L6 was more acid tolerant than L39. Three plants of each strain were grown in each container. Iron was supplied by placing the plants in a separate series of pots of distilled water containing 1 p.p.m. iron as ferric tartrate for a 6-hour period each week. The solutions were changed once a week. The lespedeza seedlings were transferred to the culture solutions on May 16, 1947 and harvested June 17, 1947. The weather was relatively cool and cloudy during the growing period.

Soybean and cowpea experiment. Three plants of Richland variety soybeans and three of Clay variety cowpeas were grown in each container in this experiment. Iron was supplied by placing the plants in a separate series of pots, containing 1 p.p.m. iron as ferric tartrate, for one 6-hour period each week during the first two weeks of the experiment. Iron was supplied twice a week during the latter half of the experiment because of the rapid growth of the plants. Solutions were changed once a week for the first two weeks and then once every five days for the remainder of the experiment. Plants were transferred to culture solutions on June 19, 1947 and harvested July 16, 1947. Soybean cotyledons were

[1]Seeds were obtained from Dr. C. P. Wilsie, Research Professor of Farm Crops, Iowa Agricultural Experiment Station.

removed at the time of transfer.

Peanut experiment. In this experiment 0.2 p.p.m. iron as ferrous sulfate was added to the culture solutions. Solutions were changed every four days. Iron was determined midway between solution changes and brought up to the original concentration. Three plants of G.F.A. Spanish variety peanuts were grown in each container. The plants were transferred to the culture solutions on September 5, 1947 and harvested October 4, 1947. Cotyledons were removed from the plants at the time of transfer.

Sweet clover experiment. The procedure used here in supplying iron and changing solutions was identical to that used in the peanut experiment. Six plants of Madrid yellow variety sweet clover were grown in each container. The plants were transferred to the culture solutions August 19, 1947 and harvested October 9, 1947.

Analytical Procedure

Harvested plants were separated into tops and roots and oven-dried to constant weight at 100°C before weighing.

Laboratory analyses

For chemical analyses, tops of the entire harvested plant sample were placed in a Pyrex beaker and ashed by the nitric-perchloric acid method (13). Manganese was oxidized to permanganate with potassium periodate and determined colorimetrically. Phosphorus was determined colorimetrically by the Truog-Meyer method (56). Calcium was precipitated as the oxalate and titrated with potassium permanganate. Iron was determined colorimetrically by the ortho-phenanthroline method (48).

Results and Discussion

Lespedeza experiment

Yields of lespedeza tops and roots grown in culture solutions of varying concentrations of manganese are given in Table 1. Marked toxicity was observed at a concentration of 1.0 p.p.m. manganese with both strains of lespedeza. The average yield with 5 p.p.m. manganese in solution was only 37 per cent of that with 0.1 p.p.m. manganese. Relative growth of plants in the low and high manganese concentrations are shown in Fig. 2. Strain L39 was more severely injured by manganese than strain L6 as shown by the relative yields. Root growth was proportional to top growth, showing no abnormality other than a brownish discoloration at the higher manganese concentrations.

Typical leaves of strain L6 from each treatment are shown in Fig. 3. Plants grown in the check solution (0.1 p.p.m. Mn) were normal in every respect and made excellent growth. Toxicity symptoms consisted of dark, reddish-brown leaf spots in

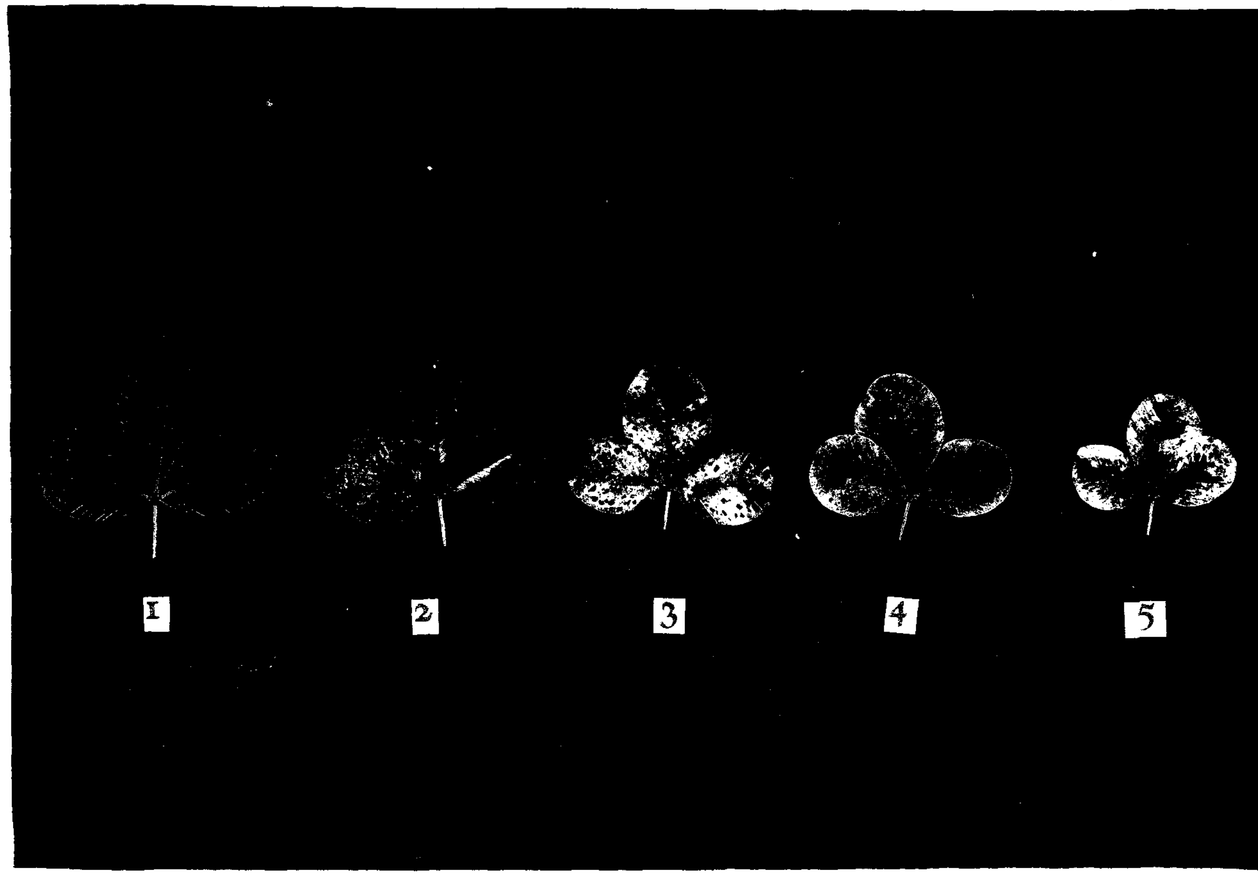


Fig. 3. Manganese toxicity symptoms of lespedeza.

No.	ppm Mn in soln.
1	0.1
2	1.0
3	2.5
4	5.0
5	10.0

Cultures 2-5, increasing in severity with the manganese level. The spots were very distinct on the under side of the leaves, being distributed in more severe cases over as much as 50 per cent of the leaf area. Considerable shedding of the more severely affected leaves was noted which may have affected the yields slightly. Leaf margins of plants grown in concentrations of 5 and 10 p.p.m. manganese were chlorotic. Approximately 75 per cent of the leaf margin was affected in the more severe cases, only a small area on either side of the stem being unaffected. Toxicity symptoms of both strains were identical.

Table 1. The effect of manganese concentration in culture solutions on the yield of lespedeza

(3 plants of each strain per pot)

Treatment number	Manganese in solution	Average yield of lespedeza per pot[1] (Oven-dry basis) Strain L6	Strain L39	Total	Relative Strain L6	Relative Strain L39	Relative Total
	ppm	gms.	gms.	gms.	%	%	%
			Tops				
1	0.1	0.82	0.98	1.80	100	100	100
2	1.0	0.60	0.58	1.18	73	59	66
3	2.5	0.48	0.49	0.97	59	50	54
4	5.0	0.38	0.28	0.66	46	28	37
5	10.0	0.33	0.22	0.55	40	22	31
			Roots				
1	0.1	0.27	0.25	0.52	100	100	100
2	1.0	0.21	0.18	0.39	78	72	75
3	2.5	0.15	0.15	0.30	56	60	58
4	5.0	0.13	0.08	0.21	48	32	40
5	10.0	0.09	0.05	0.14	33	20	27

[1] Average of 3 replications.

Table 2. The effect of manganese concentration in culture solutions on manganese, calcium, and iron content of lespedeza

Treatment number	Manganese in solution	Plant composition (tops)								
		Manganese			Calcium			Iron		
		Strain		Average	Strain		Average	Strain		Average
		L6	L39		L6	L39		L6	L39	
	ppm	ppm	ppm	ppm	%	%	%	ppm	ppm	ppm
1	0.1	133	130	131	1.01	1.02	1.02	193	193	193
2	1.0	608	530	570	0.96	1.13	1.04	172	209	190
3	2.5	1455	1351	1402	1.03	1.12	1.08	264	289	277
4	5.0	2941	2875	2913	1.10	1.29	1.18	225	339	273
5	10.0	6000	3750	5100	1.10	1.33	1.19	216	300	250

Calcium, manganese, and iron analyses of both strains of lespedeza grown in all cultures are given in Table 2. Manganese was readily absorbed by both strains, the concentrations in the plant being roughly proportional to the amount of manganese in solution. No appreciable differences were found in the manganese contents of the two strains except at the highest manganese level where L6 contained a much higher concentration. Calcium absorption was augmented apparently by increasing the concentration of manganese in the nutrient solution. This, however, may be more apparent than real, probably being due to the relatively high ash content of poor, slow growing plants as compared to that of healthy, vigorously growing plants. Strain L39 contained more calcium than L6 in all treatments, especially in Cultures 4 and 5. Strain L39 was also found to contain higher amounts of calcium when the two strains were grown in soil. Apparently, the relatively better growth of strain L6 than strain L39 in acid soils may be attributed, at least in part, to its greater tolerance to soluble manganese and to its lower requirement for calcium. Iron contents of both strains were somewhat erratic but the trend was toward a higher concentration of iron with increased concentrations of manganese in solution. The total iron content of the plants indicated that sufficient iron was supplied the plants for normal growth.

Soybean and cowpea experiment

Yields, manganese and calcium contents of soybeans and cowpeas grown in culture solutions of varying manganese concentrations are given in Table 3. Although both legumes exhibited toxicity symptoms at manganese concentrations as low as 2.5 p.p.m. it was only at the highest manganese level, 10 p.p.m., that a marked decrease in growth resulted. Relative yields indicate that both species were affected approximately in the same degree by increasing concentrations of manganese. Both legumes appeared to be more severely injured by manganese during early growth, apparently becoming more resistant to manganese toxicity with age. Cowpeas and soybeans growing in the culture solution of pH 6.0 made poor growth and exhibited symptoms of iron deficiency. Some improvement in color and growth of plants in this culture was noted during the last week of the growing period.

Roots of both species were apparently not directly injured by manganese, being normal in appearance and proportional to top growth in weight.

The calcium and manganese contents of cowpeas were much higher in all cases than that of soybeans grown in the same culture solution. More calcium and manganese were found to be absorbed by both species at pH 6.0 than at pH 4.6 which agrees with previously reported data (4, 38).

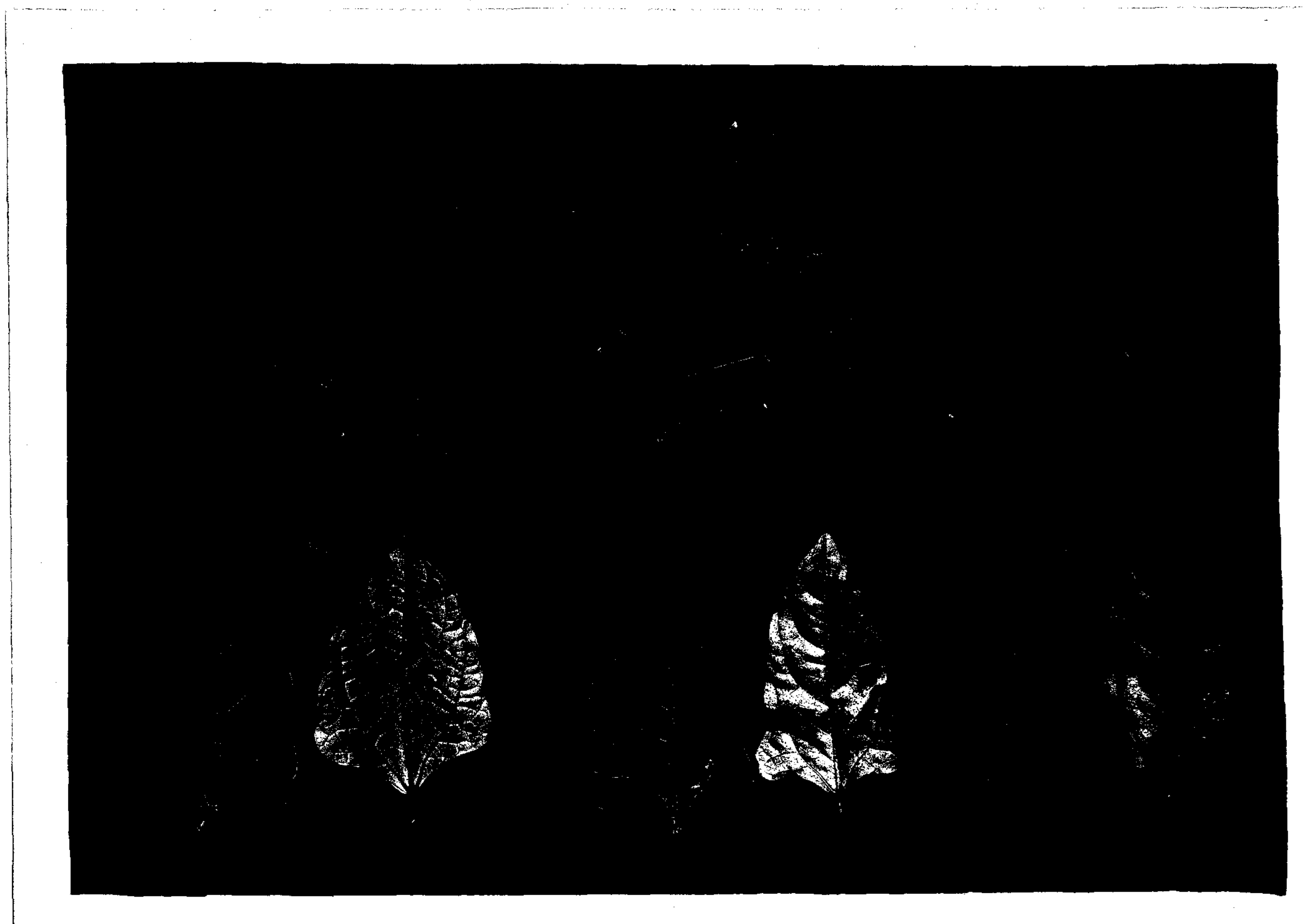
Table 3. Yield, manganese and calcium content of soybeans and cowpeas (tops) grown in culture solutions of different manganese concentrations

(3 plants of each legume per pot)

Treatment Number	Solution culture pH	Solution culture Mn	Average yield per pot[1] Soybeans		Average yield per pot[1] Cowpeas		Plant composition Manganese Soybeans	Plant composition Manganese Cowpeas	Plant composition Calcium Soybeans	Plant composition Calcium Cowpeas
		ppm	gms.	%	gms.	%	ppm	ppm	%	%
0	6.0	0.1	3.08	45	4.26	61	143	219	1.69	3.26
1	4.6	0.1	6.84	100	6.97	100	40	74	1.39	2.59
2	4.6	1.0	6.17	90	6.10	86	266	615	1.66	2.82
3	4.6	2.5	6.89	101	6.56	94	529	1224	1.52	2.80
4	4.6	5.0	6.35	93	6.11	88	1040	2292	1.45	2.62
5	4.6	10.0	4.13	60	4.72	68	2168	4212	1.67	2.72

[1] Average of 2 replications.

Manganese toxicity symptoms of soybeans and cowpeas were entirely different (Fig. 4). Neither exhibited the chlorotic leaf margin observed in lespedeza at the higher manganese



0.1 ppm Mn 2.5 ppm Mn 10 ppm Mn

Top - soybeans Bottom - cowpeas

Fig. 4. Manganese toxicity symptoms of soybeans and cowpeas grown in culture solutions of different manganese concentrations.

concentrations. Soybean symptoms consisted of pale green irregular areas between the main veins of the leaves. The number of leaves affected, and the leaf area occupied by these irregular areas increased with the manganese concentration of the culture solutions. The percentage of leaves affected varied from approximately 25 per cent with 2.5 p.p.m. in the culture solution to 100 per cent at the 10 p.p.m. manganese level. The affected areas became brown and died, especially with the higher levels of manganese. New leaves of plants growing in the 10 p.p.m. manganese culture were crumpled and distorted and definitely smaller than leaves of plants in the cultures containing less manganese.

Cowpea toxicity symptoms consisted of small reddish-purple spots distributed uniformly over the leaf area. These spots were also distinctly visible on the under side of the leaves. The number of spots per leaf increased with the manganese concentration of the culture solution. New leaves were normal and the spots developed with age. Approximately 25 per cent of the older leaves were affected in the 2.5 p.p.m. manganese treatment and 100 per cent of the older leaves in the 10 p.p.m. manganese solution.

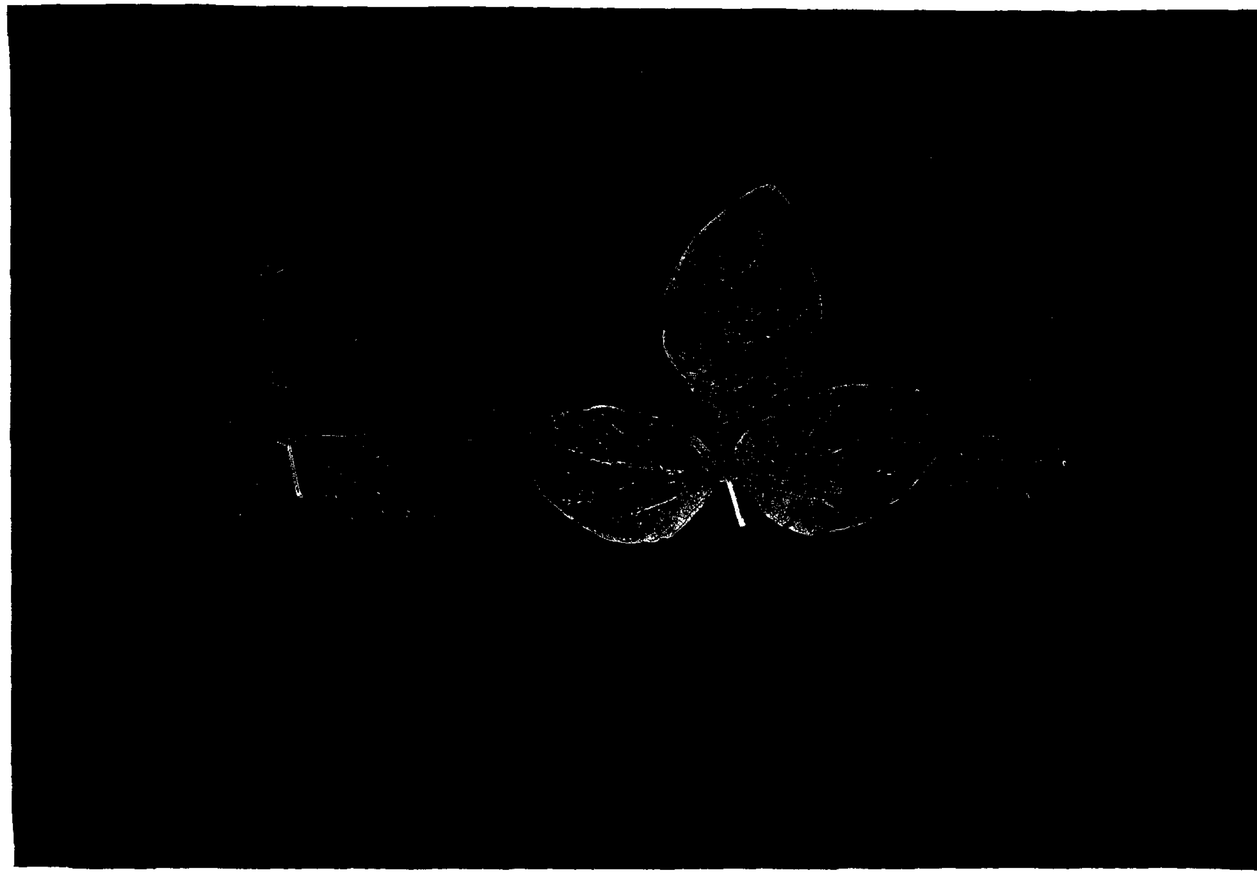
Chlorosis of the plants grown in the pH 6.0 culture solution was entirely different from those of plants grown in the high manganese culture colutions. Typical soybean leaves from

Cultures 0, 1, and 6 are given in Fig. 5. To confirm the fact that the symptoms exhibited by plants in the pH 6.0 culture were due to iron deficiency, a series of observational pots were set up. The standard nutrient solution was employed and solutions were changed every two days. The iron and manganese concentrations of the solutions are given in Table 4.

Table 4. Iron and manganese concentrations of culture solutions used in soybean deficiency and toxicity symptoms study

Treatment number	Solution Fe ppm	Culture Mn ppm	Theoretical symptom
1	0.2	0.1	None
2	0.2	0.0	Manganese deficiency
3	0.2	10.0	Manganese toxicity
4	0.0	0.1	Iron deficiency
5	3.0	0.1	Iron toxicity

Typical leaves from Treatments 1 to 4 are shown in Fig. 6. Plants in Treatment 5 were normal and leaves from that treatment are not included. It is evident that iron deficiency symptoms as produced under these conditions are not identical with manganese toxicity symptoms. As no symptoms of iron toxicity were produced, despite the iron-manganese ratio of 30:1 used in Treatment 5, no comparison could be made between iron toxicity and manganese toxicity symptoms. The iron deficiency



pH 4.6 Mn 0.1 ppm | pH 6.0 Mn 0.1 ppm | pH 4.6 Mn 10 ppm

Fig. 5. Typical soybean leaves from culture solutions of different manganese and hydrogen-ion concentrations.

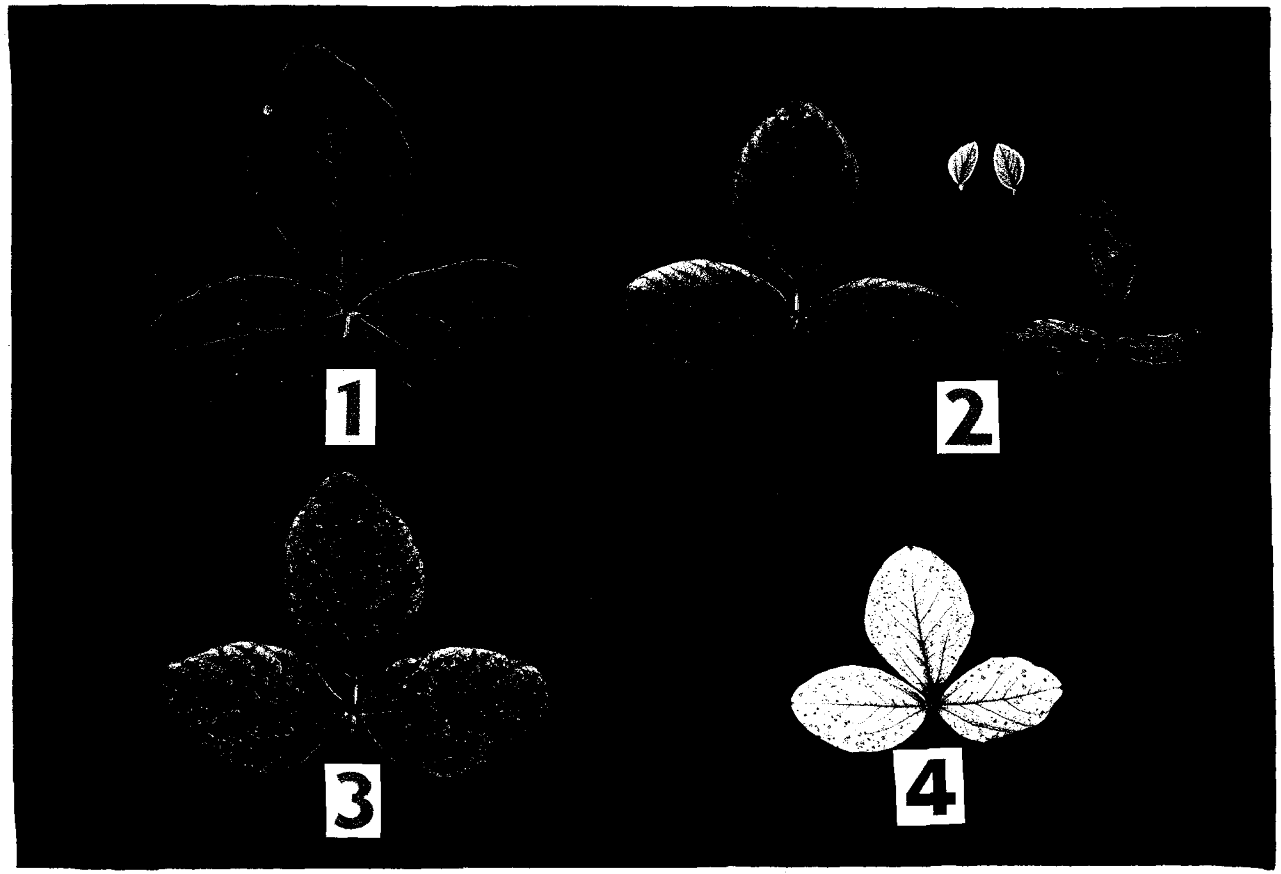


Fig. 6. Toxicity and deficiency symptoms of soybeans.
1. Normal 2. Manganese deficiency
3. Manganese toxicity 4. Iron deficiency

symptoms observed here were similar to, but much more severe than those found in soybeans grown in the pH 6.0 culture solutions.

Peanut experiment

Results of this experiment are given in Table 5. The plants grew well in all manganese concentrations and a reduction in growth occurred only with the highest manganese concentration, 10 p.p.m. Toxicity symptoms were slight even in this culture, only a small percentage of the leaves showing a slight chlorosis of the leaf margin. The chlorosis appeared two weeks after the plants were placed in the culture solutions and had almost entirely disappeared at harvest. Roots were slightly browner at the two higher manganese levels.

Table 5. Yield, manganese and calcium content of peanuts grown in culture solutions of different manganese concentrations (3 plants per pot)

Treatment number	Solution culture pH	Solution culture Mn	Average yield per pot[1] (tops)		Plant composition (tops) Manganese	Plant composition (tops) Calcium
		ppm	gms	%	ppm	%
1	4.6	0.1	7.36	100	49	1.43
2	4.6	1.0	6.93	94	200	1.42
3	4.6	2.5	7.01	95	378	1.32
4	4.6	5.0	6.91	94	656	1.24
5	4.6	10.0	5.56	76	1245	1.10

[1] Average of 3 replications.

The manganese content of the plants grown in any given concentration of manganese was relatively low as compared to the other legumes. This factor may be largely responsible for the tolerance of peanuts to soluble manganese. The calcium content of the plants decreased markedly as the manganese concentration of culture solutions increased.

<u>Sweet clover experiment</u>

Sweet clover was slow to become established in the culture solutions but at harvest the plants in most of the cultures were making excellent growth. Plants in the pH 6.0 culture grew as well but not better than those in the more acid solutions containing low concentrations of manganese. Sweet clover was not injured therefore by the strong acidity of the culture solution.

Yield, manganese and calcium contents of sweet clover are given in Table 6. The roots of one replicate of Treatment 1 were diseased and the resulting poor growth was responsible for the low average yield of this treatment.

Manganese toxicity symptoms were observed in plants grown in culture solutions containing 2.5 and 5 p.p.m. manganese. Symptoms were a marked chlorosis of the distal leaf margin usually accompanied by a definite crimping of the leaf. No spotting was evident. Typical leaves from three treatments are shown in Fig. 7.

Only 321 p.p.m. manganese were found in the plants grown in Culture 3, containing 2.5 p.p.m. manganese, even though toxicity symptoms were evident. Peanuts grown in solutions of the same manganese concentration contained approximately the same amount of manganese but were not injured. Therefore, at least two factors affect the tolerance of a plant to soluble manganese; the amount of manganese absorbed by the plant, and the relative ability to endure large amounts of manganese within the plant.

Table 6. Yield, manganese and calcium content of sweet clover grown in culture solutions of different manganese concentrations

(6 plants per pot)

Treatment number	Solution culture		Average yield per pot[1] (tops)		Plant composition (tops)	
	pH	Mn			Manganese	Calcium
		ppm	gms.	%	ppm	%
1	6.0	0.1	5.82	107	64	1.49
2	4.6	0.1	5.43	100	27	1.05
3	4.6	1.0	6.45	119	158	1.10
4	4.6	2.5	4.80	88	321	0.97
5	4.6	5.0	4.19	77	754	1.09

[1] Average of 3 replications.

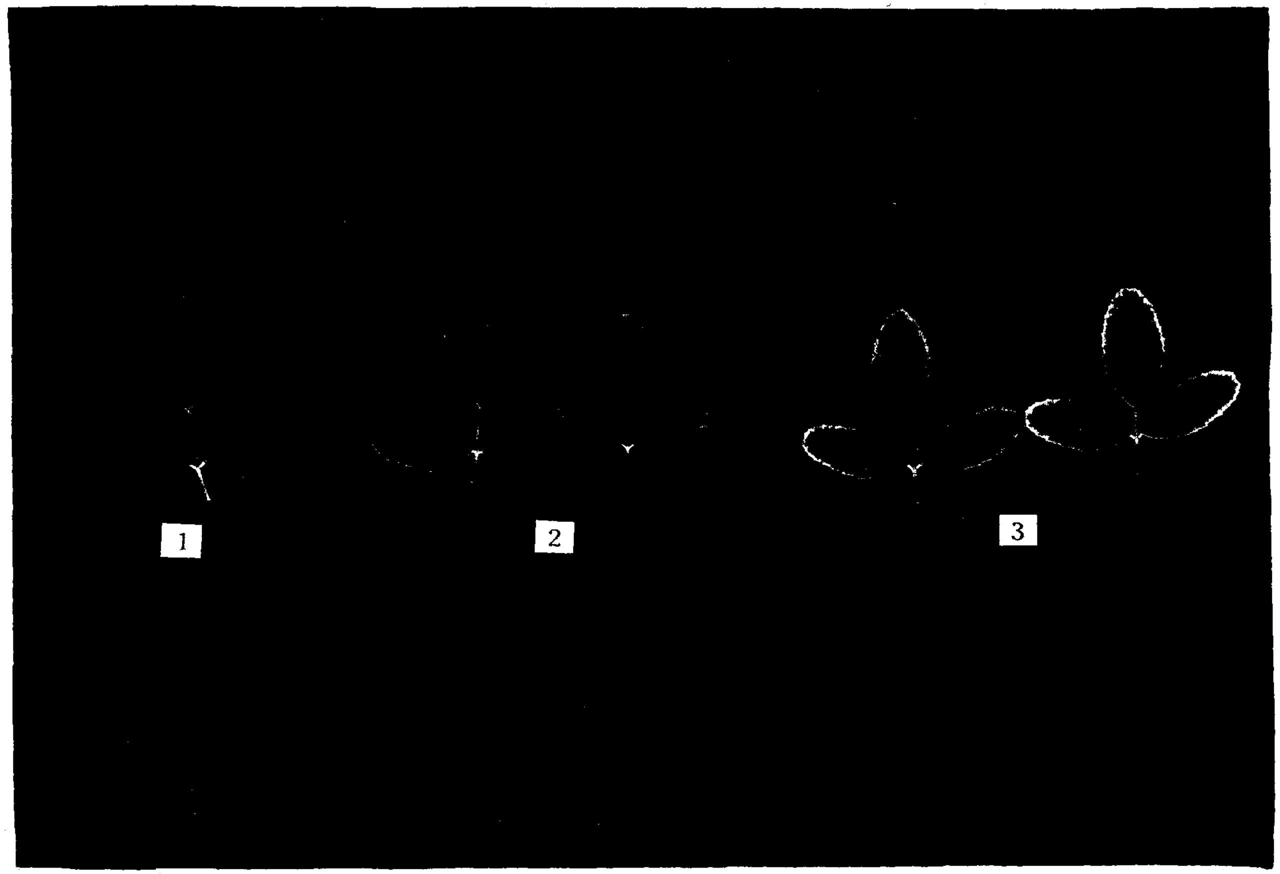


Fig. 7. Manganese toxicity symptoms of sweet clover grown in culture solutions of different manganese concentrations.

No.	ppm Mn in soln.
1	0.1
2	2.5
3	5.0

THE EFFECT OF VARIOUS CONCENTRATIONS OF OTHER NUTRITIVE ELEMENTS ON THE TOLERANCE OF LESPEDEZA TO MANGANESE TOXICITY

Introduction

Lespedeza is a legume of considerable economic importance. It is quite commonly grown on rather acid soils where relatively high concentrations of water-soluble manganese might occur. Under such acid conditions available calcium and phosphorus are usually deficient and may tend to increase the degree of toxicity resulting from a given concentration of manganese.

The specific objective of this investigation was to determine the effect of various concentrations of calcium, phosphorus, and iron in culture solutions on the toxicity of manganese to lespedeza.

Experimental

Plan and procedure

This investigation was carried out in three experiments, A, B, and C, in which the effects of calcium, phosphorus, and iron, respectively, were studied. With few exceptions the culture solution and general procedure were identical to those used in the previous manganese tolerance experiments with various legumes.

In these experiments 0.2 p.p.m. iron was supplied continuously in culture solutions as ferrous sulfate, except in Experiment C where varying concentrations of iron were used. All calcium in Experiment A was supplied as calcium sulfate. Since calcium nitrate was omitted, the nitrogen in this experiment was decreased to 28 p.p.m. Solutions were changed every four days and iron determined midway between changes of solutions and brought up to the original concentration. Manganese concentrations were checked and found to remain constant. Phosphorus determinations were made at frequent intervals but in no case was the phosphorus concentration lowered more than 10 per cent or enough to necessitate addition of phosphate. The reaction of the solutions remained fairly constant, seldom becoming more acid than pH 4.4.

All experiments, except Experiment C-2, were of factorial design consisting of three levels of calcium and three levels of manganese in Experiment A, two levels of phosphorus and two levels of manganese in Experiment B, and two levels of iron and three of manganese in Experiment C-1.

Four plants each of Strain L6 and Strain L39 were grown in each container, except in Experiment C-2 where eight of Strain L6 alone were grown. Treatments were replicated twice in

Experiments A and C-1; in other experiments they were replicated three times. Growing periods were July 6 to August 15, 1947 for Experiments A and C-1; August 9 to September 4, 1947 for Experiment B; and September 14 to October 17, 1947 for Experiment C-2.

Analytical procedures were identical with those reported previously for the legume tolerance experiment.

Results and Discussion

Experiment A - Effect of calcium concentration on manganese toxicity

Yields and chemical composition of both strains of lespedeza grown in culture solutions of three levels of calcium in all combinations with three levels of manganese are given in Table 7. One additional treatment at each calcium level was included to study the effect of maintaining a hydrogen-ion concentration of pH 6.0 on the growth of lespedeza.

Plants grown at pH 6.0 were stunted and very chlorotic, showing typical iron deficiency symptoms. To confirm the conclusion that the symptoms observed in these treatments were due to iron deficiency, an observational culture of pH 4.6 was set up in which iron was omitted entirely from the solution. Plants grown in this solution rapidly developed symptoms identical with those grown at pH 6.0. A comparison of manganese toxicity and iron deficiency symptoms is shown in Fig. 8. The average con-

Table 7. The yield and chemical composition of lespedeza (tops) grown in culture solutions of varying calcium and manganese concentrations

(4 plants of each strain per pot)

Treatment number	Culture solution			Average yield of lespedeza * per pot. (oven-dry basis)			Plant composition					
				Strain		Relative average of both strains	Calcium		Manganese		Iron	
							Strain		Strain		Strain	
	pH	Ca	Mn	L6	L39		L6	L39	L6	L39	L6	L39
		ppm	ppm	gms.	gms.	%	%	%	ppm	ppm	ppm	ppm
1	6.0	12	0.1	0.09	0.10	15	1.24	1.51	113	139	677	611
2	4.6	12	0.1	0.64	0.62	100	0.65	0.62	83	82	526	630
3	4.6	12	1.0	0.62	0.74	108	0.62	0.62	567	522	438	464
4	4.6	12	5.0	0.20	0.11	25	0.74	0.94	3378	3056	784	1028
5	6.0	60	0.1	0.14	0.16	13	1.62	1.90	123	161	509	518
6	4.6	60	0.1	1.06	1.17	100	1.17	1.22	42	44	301	318
7	4.6	60	1.0	0.97	1.06	91	1.29	1.26	495	413	295	323
8	4.6	60	5.0	0.24	0.17	18	1.35	1.51	2794	2403	600	726
9	6.0	300	0.1	0.16	0.16	24	2.59	2.93	121	120	629	685
10	4.6	300	0.1	0.57	0.79	100	2.00	2.03	60	31	375	292
11	4.6	300	1.0	0.48	0.50	72	2.07	2.34	506	435	574	1081
12	4.6	300	5.0	0.22	0.10	24	2.21	2.64	2402	2250	500	986

* Average of 2 replications.

centration of iron found in the pH 6.0 solution when checked for adjustment was 0.12 p.p.m. compared to 0.15 p.p.m. for the more acid solutions. The high iron content of the plants grown at pH 6.0 indicates that chlorosis resulted from an immobility of iron within the plant rather than a deficiency of available iron in the culture solution.

Manganese concentrations of 5 p.p.m. were extremely toxic regardless of the calcium concentration. Growth of lespedeza at different levels of calcium with low and high manganese is shown in Fig. 9. It is evident that manganese toxicity is not alleviated by additional calcium in the case of lespedeza; in fact there is some indication that with the medium level of manganese, 1.0 p.p.m., the toxicity was greater at the higher calcium concentration.

Optimum growth of lespedeza was obtained in the 60 p.p.m. calcium concentrations. Increasing the calcium concentration to 300 p.p.m. resulted in a reduction in growth at all manganese levels, probably due to the unbalanced nutrient solution. Plants in the low calcium and low manganese solutions grew well and were apparently normal although containing very low amounts of calcium as compared to the plants grown at higher calcium levels.

Experiment B - Effect of phosphorus concentration on manganese toxicity

Yield data from the experiment are given in Table 8.

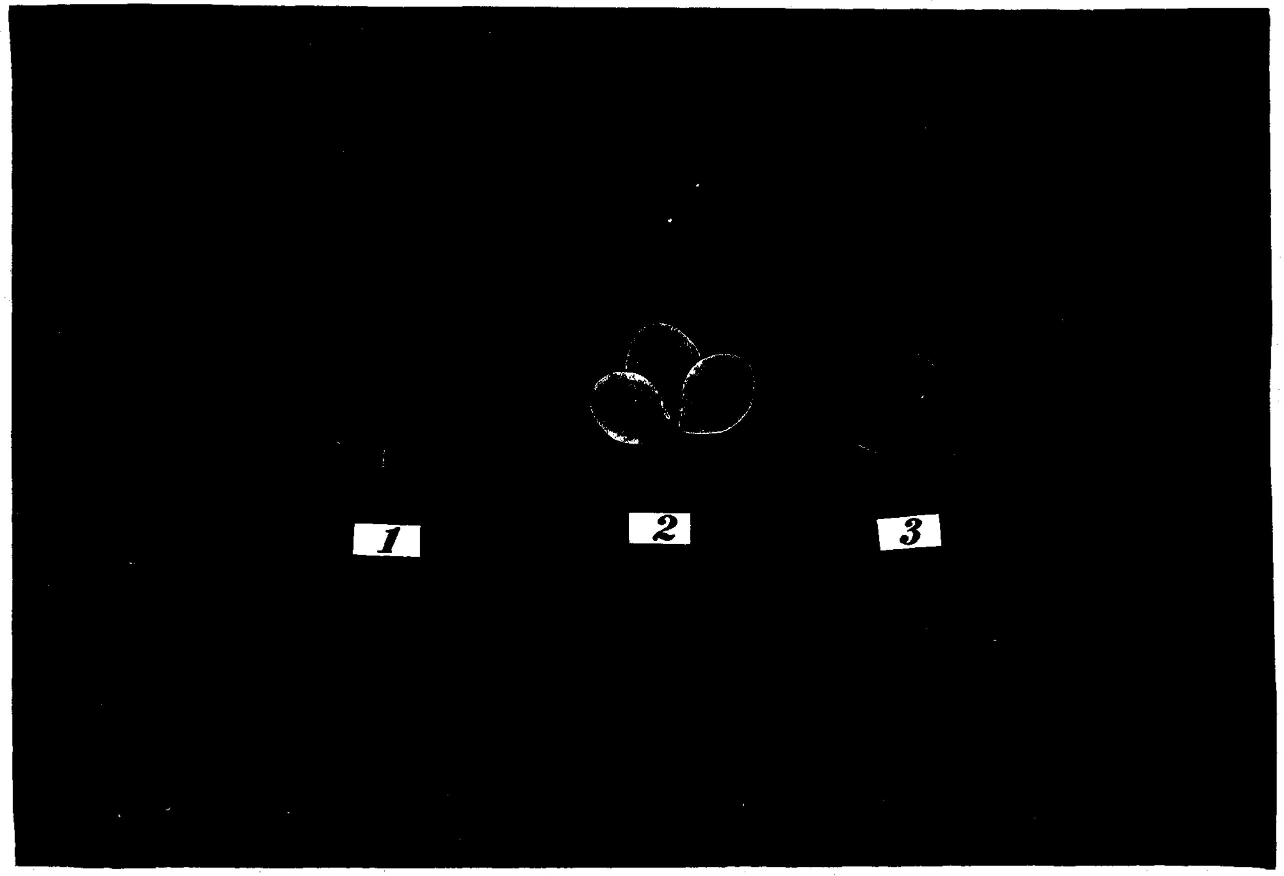


Fig. 8. Manganese toxicity and iron deficiency symptoms of lespedeza.
1. Normal 2. Iron deficiency 3. Manganese toxicity (5ppm)

	5	6	7	8
pH	6.0	4.6	4.6	4.6
Ca(ppm)	60	60	60	60
Mn(ppm)	0.1	0.1	1.0	5.0

	2	4	6	8	10	12
Ca(ppm)	12	12	60	60	300	300
Mn(ppm)	0.1	5.0	0.1	5.0	0.1	5.0

Fig. 9. Growth of lespedeza in culture solutions of varying calcium and manganese concentrations.

Table 8. Yield of lespedeza (tops)grown in culture solutions of different phosphorus and manganese concentrations

(4 plants of each strain per pot)

Treat-ment number	Culture solution		Average yield of lespedeza per pot[1] (oven-dry basis)					
			Strain			Relative		
						Strain		
	P	Mn	L6	L39	Total	L6	L39	Total
	ppm	ppm	gms.	gms.	gms.	%	%	%
1	2	0.1	1.01	0.51	1.52	100	100	100
2	2	2.5-5.0[a]	0.69	0.64	1.33	68	125	88
3	20	0.1	0.72	0.73	1.45	100	100	100
4	20	2.5-5.0[a]	0.34	0.33	0.67	47	45	46

[1]Average of 3 replications.
[a]Received 2.5 p.p.m. Mn for first half of experiment and 5.0 p.p.m. Mn for remainder of experiment.

Strain L39 made rather poor growth in all replicates of Culture 1 and the yield was lower than that obtained in Culture 2 for that strain. Only a moderate 12 per cent reduction in growth was obtained from the high manganese concentration with low phosphorus (2 p.p.m.). However, with the high phosphorus (20 p.p.m.) and high manganese a decrease in growth of 54 per cent was obtained. The average concentrations of iron found in the solutions before adjustment were 0.13 p.p.m. with the low phosphorus concentration, and 0.11 p.p.m. for the high phosphorus. Although the slightly lower amount of iron remaining in solution at the high phosphorus level may partly account for the greater toxicity resulting from manganese in Culture 4, this does not seem to be

Table 9. Chemical composition of lespedeza grown in culture solutions of different phosphorus and manganese concentrations

Treat-ment no.	Culture solution		Plant composition							
			P		Ca		Mn		Fe	
			Strain		Strain		Strain		Strain	
	P	Mn	L6	L39	L6	L39	L6	L39	L6	L39
	ppm	ppm	%	%	%	%	ppm	ppm	ppm	ppm
					Leaves					
1	2	0.1	0.39	0.39	1.54	1.61	22	<20	178	213
2	2	2.5-5.0[1]	0.36	0.39	1.71	1.70	1653	1418	333	190
3	20	0.1	0.42	0.47	1.89	1.91	59	36	191	164
4	20	2.5-5.0[1]	0.35	0.38	1.52	1.63	1698	1500	247	202
					Stems					
1	2	0.1	0.35	0.41	0.98	1.24	<20	<20	208	247
2	2	2.5-5.0[1]	0.44	0.48	1.25	1.49	506	450	224	275
3	20	0.1	0.44	0.51	1.26	1.18	<20	<20	211	213
4	20	2.5-5.0[1]	0.48	0.51	1.44	1.64	677	661	298	265

[1]Received 2.5 p.p.m. Mn for first half of experiment and 5.0 p.p.m. Mn for remainder of experiment.

very probable. The solubility of manganese in the culture solutions was not affected by the additional phosphate. Toxicity symptoms appeared on plants in Culture 4 earlier than in Culture 2 and were more pronounced. Typical leaves from each treatment are shown in Fig. 10.

Analyses of both leaves and stems were made to ascertain if iron or manganese was precipitated in the stems of the plants at the higher phosphorus concentration. No evidence was found that precipitation of either element occurred (Table 9). Increasing

	1	2	3	4
P(ppm)	2	2	20	20
Mn(ppm)	0.1	5.0	0.1	5.0

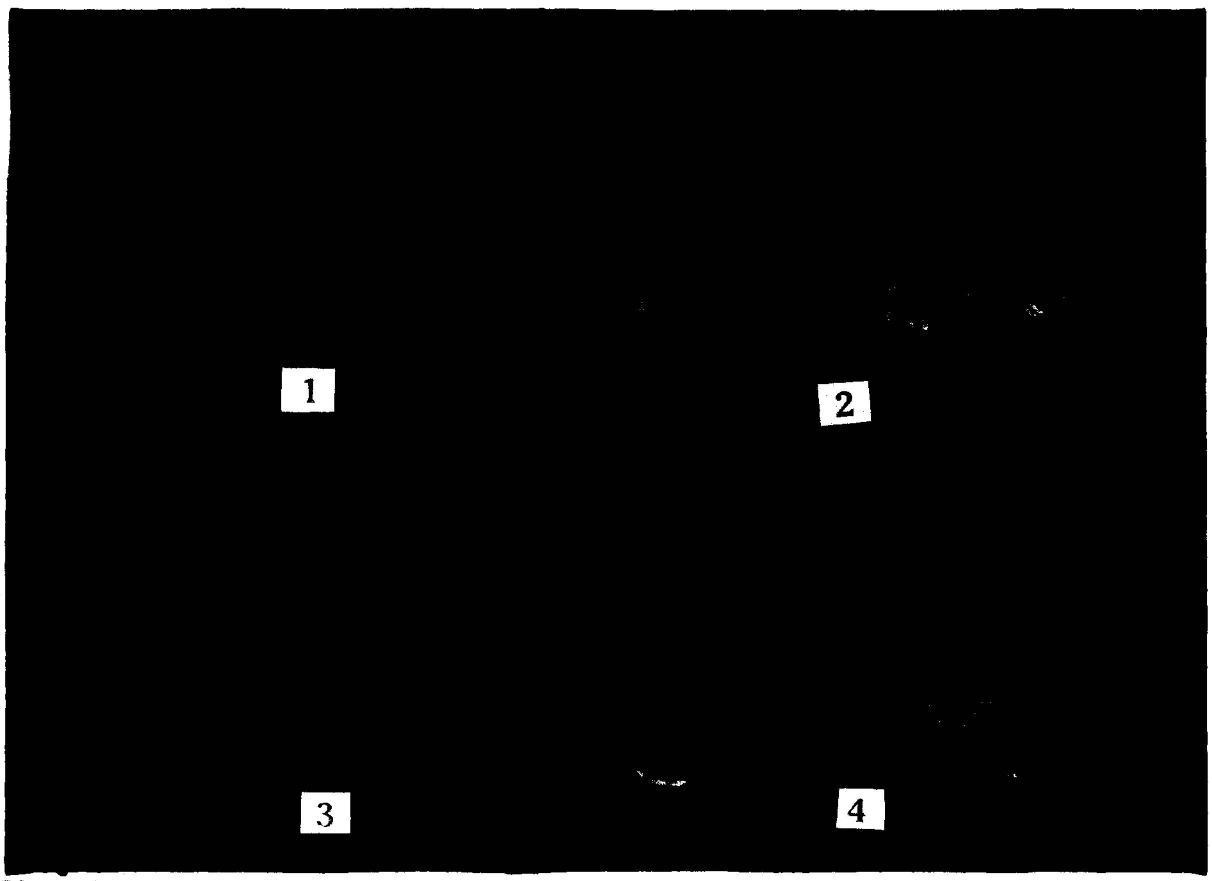


Fig. 10. Growth and manganese toxicity symptoms of lespedeza grown in culture solutions of different phosphorus and manganese concentrations.

the phosphorus concentration of the culture solution from 2 to 20 p.p.m., increased the manganese content of the plant slightly but had no appreciable effect on the phosphorus, calcium or iron content. Cultures 2 and 4 received 2.5 p.p.m. manganese during the first half of the experiment in order to obtain a fair amount of growth and then the manganese concentration was increased to 5 p.p.m. in these cultures.

The high phosphorus content of the plants grown at the 2 p.p.m. phosphorus concentrations indicates that phosphorus was more than sufficient for optimum growth of lespedeza even at the 2 p.p.m. level. Nevertheless, it is clearly evident that low concentrations of manganese are toxic to lespedeza regardless of the concentration of phosphorus present.

Experiment C - Effect of iron concentration on manganese toxicity

The concentrations of iron used in the first part of this experiment were 0.2 and 1.0 p.p.m., each in combination with the three levels of manganese, 0.1, 1.0, and 5.0 p.p.m. Much better growth was obtained with the higher rate of iron as shown in Table 10. Root systems of the plants grown in the 1 p.p.m. iron solutions were larger, lighter in color, and contained more new growth at the end of the experiment than those in the lower iron solutions. Plants grown in the 1 p.p.m. manganese solutions showed slight toxicity symptoms, the older

Table 10. Yield and chemical composition of lespedeza (tops) grown in culture solutions of different iron and manganese concentrations

(4 plants of each strain per pot)

Treatment number	Culture solution		Average yield of lespedeza per pot* (oven-dry basis)				Plant composition					
			Strain		Total		Calcium Strain		Manganese Strain		Iron Strain	
	Fe	Mn	L6	L39			L6	L39	L6	L39	L6	L39
	ppm	ppm	gms.	gms.	gms.	%	%	%	ppm	ppm	ppm	ppm
13	0.2	0.1	0.44	0.56	1.00	100	1.37	1.29	95	82	459	380
14	0.2	1.0	0.46	0.64	1.10	110	1.46	1.48	445	379	439	361
15	0.2	5.0	0.26	0.18	0.44	44	1.48	1.60	1957	1879	478	515
16	1.0	0.1	0.82	0.91	1.73	100	1.29	1.46	40	47	443	345
17	1.0	1.0	0.89	0.68	1.57	91	1.20	1.42	192	190	396	438
18	1.0	5.0	0.64	0.54	1.18	68	1.25	1.44	769	792	325	312

*Average of 2 replications.

leaves being slightly spotted, but the growth was not reduced at either iron level. However, at the high manganese level of 5 p.p.m. a marked difference was noticeable between the plants grown in the different iron solutions. Plants grown in the low iron solutions were stunted and exhibited marked manganese toxicity symptoms whereas the plants in the high iron solution grew well and showed only moderate injury in the form of spotted older leaves.

Plant analyses also given in Table 10 show that the alleviation of the manganese toxicity by the higher iron concentration was not due to an increase of total iron in the plant but to an approximate 50 per cent reduction in the manganese content of the plants. Therefore, the beneficial action of iron in reducing manganese toxicity appears to be due to some mechanism which prevents the excessive absorption and subsequent accumulation of manganese in the plant rather than to a corrective action of iron within the plant itself.

While results from this experiment showed clearly that iron was effective in reducing manganese toxicity of lespedeza, they failed to support Somers and Shive's (54) theory that the iron-manganese ratio of the culture solutions was the factor controlling plant growth rather than the total amounts of these elements present. For further study of this point a second experiment was carried out in which lespedeza was grown in culture solutions of somewhat different iron-manganese ratios. Two of the ratios were duplicated with higher total amounts of iron and manganese. Treatments used in this experiment, toxicity symptoms, yields, and chemical composition of lespedeza are given in Table 11. Typical leaves from each treatment are shown in Fig. 11.

Plants grown in Culture 3 with 0.5 p.p.m. iron and 1 p.p.m. manganese made excellent growth and were much larger than plants grown in the other treatments. Some slight spotting of the leaves were observed even in this culture. Plants grown in Culture 6, with the same iron-manganese ratio as in Culture 3 but with five times as much total iron and manganese, made poor growth and exhibited severe manganese toxicity symptoms. The greater toxicity symptoms and reduced growth resulting from 5 p.p.m. manganese with 0.5 or 2.5 p.p.m. iron in this experiment, as compared to the same rate of manganese with 1 p.p.m. iron in the previous experiment, may be due to the greater light intensity prevailing during the second experiment, for it has been shown by McCool (35) that manganese toxicity is greater at high light intensities.

No explanation is offered for the poor growth of plants in Culture 5. The phosphorus content is lower than in plants grown in the other solutions but much higher than the phosphorus content usually found in lespedeza grown in soil. The iron content of the plants is approximately double that found in plants grown

Table 11. Yield and chemical composition of lespedeza (tops) grown in culture solutions of different iron-manganese ratios

(8 plants of Strain 76 per pot)

Treatment number	Culture solution			Average yield lespedeza per pot* (oven-dry basis)	Toxicity symptoms	Plant composition			
	Fe	Mn	Fe/Mn ratio			P	Ca	Mn	Fe
	ppm	ppm		gms.		%	%	ppm	ppm
1	0.1	0.1	1:1	1.93	Very slight spotting	0.43	0.95	70	130
2	0.1	1.0	1:10	1.41	Severe spotting	0.46	1.20	826	144
3	0.5	1.0	1:2	2.84	Slight spotting	0.45	0.88	133	158
4	0.5	5.0	1:10	1.76	Severe spotting	0.51	1.00	719	180
5	2.5	1.0	2.5:1	1.71	Very slight spotting	0.36	1.00	131	327
6	2.5	5.0	1:2	1.53	Severe spotting	0.50	1.30	836	342

*Average of 3 replications.

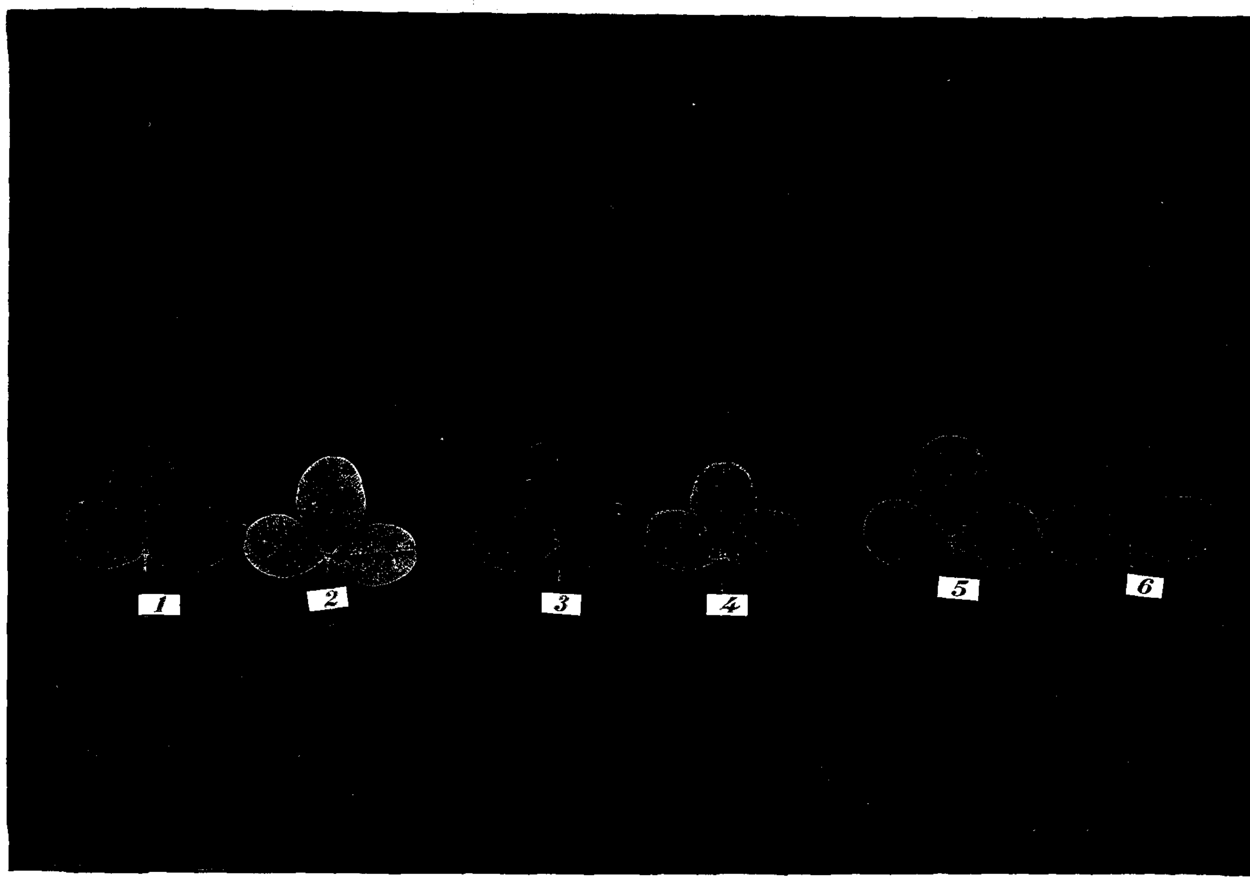


Fig. 11. Manganese toxicity symptoms of lespedeza grown in culture solutions of different iron-manganese ratios.

No.	ppm in soln. Fe	Mn	No.	ppm in soln. Fe	Mn	No.	ppm in soln. Fe	Mn
1	0.1	0.1	3	0.5	1.0	5	2.5	1.0
2	0.1	1.0	4	0.5	5.0	6	2.5	5.0

in the lower iron concentrations but no greater than that found in many cases in the earlier experiments. No symptoms were evident other than slight spotting of the leaves similar to the symptoms observed in Cultures 1 and 3.

Chemical analyses again showed that the manganese content of plants was greatly reduced by increasing the amount of iron in solution (Cultures 2 and 3), even though the iron content of the plants was increased but slightly by the additional iron. Limitations as to the use of iron in overcoming manganese toxicity are evident from the manganese content of the plants grown in Cultures 4 and 6. It is apparent from the manganese content of the plants that an increase in the iron concentration of the culture solution from 0.5 to 2.5 p.p.m. produced no additional decrease in manganese absorption.

Therefore, it appears from the data presented that a certain minimum concentration of iron in the nutrient solution is necessary for optimum growth of lespedeza regardless of the manganese concentration of the solution. The optimum iron concentration in this experiment was in the proximity of 1 p.p.m. Above this optimum a depression of growth was obtained regardless of the manganese concentration of the solution. The inconsistency of the yields and toxicity symptoms obtained with identical iron-manganese ratios but different total amounts of iron and manganese in the culture solution indicates strongly that the iron-manganese ratio is not the primary factor regulating the toxicity of manganese to lespedeza.

THE SOLUBLE MANGANESE CONTENT OF ACID SOILS AND ITS RELATION TO THE GROWTH AND MANGANESE CONTENT OF SWEET CLOVER AND LESPEDEZA

Introduction

Recent work by Arnon and associates (2, 3) has shown that acidity greater than that usually found in acid soils is not directly injurious to plant growth. These investigators found that normal plant growth could be obtained at pH 4.0 in nutrient solutions containing 280 p.p.m. calcium. Therefore, it would appear that the high amounts of calcium found in many acid prairie soils should be sufficient for normal plant growth and that poor growth of legumes occurring on these soils might be due to other injurious factors.

The purpose of this investigation was to determine if soluble manganese might be a toxic factor to plant growth on certain acid soils.

The specific objectives were: 1) To determine the concentrations of soluble manganese in various acid soils in relation to their H-ion concentrations and their contents of exchangeable manganese. 2) To study the relation between the soluble manganese content of acid soils and the growth and manganese content of sweet clover and lespedeza. 3) To determine the relative effects of equivalent amounts of calcium carbonate and calcium sulfate on the soluble manganese content of soils and on the growth and manganese content of sweet clover and lespedeza. 4) To study the effects of large amounts of phosphate fertilizer on water-soluble manganese in the soil and on the growth of lespedeza and sweet clover.

Experimental

General procedure

All the soils used in the pot experiments, with the exception of the two Cecil soils, were obtained from different locations in Iowa. The Carrington soils used in the main experiments were from the Howard County Experimental Farm. The two Cecil soils were obtained from North Carolina and Georgia.

Bulk samples of the soils for all pot experiments were taken from as uniform a location as possible, brought into the greenhouse, and allowed to air-dry. They were then passed through a 0.25-inch mesh screen, mixed thoroughly and potted. Pots were filled to within one-half inch of the top with soil. Glazed earthenware pots without drains, of one-half gallon capacity, were used in the lespedeza experiments and in approximately half of the sweet clover experiments.

Madrid yellow variety sweet clover was grown in all the sweet clover experiments. Strain L6 Korean lespedeza was used in all lespedeza experiments. Strain L39 was included in two of the lespedeza experiments to obtain information as to whether strains of the same species might differ in their ability to tolerate acid soil conditions. Four to six sweet clover plants were grown in each pot, depending on the size of the container and the length of the growing period. Lespedeza stands were thinned to eight plants per pot, except in the first Carrington soil experiment where only six plants per container were grown because of the longer growing period. Lespedeza and sweet clover were inoculated with cultures of the proper nitrogen-fixing organism in both Carrington soil experiments.

Chemically pure compounds were used in all cases as sources of calcium and other nutritive elements. Calcium carbonate and calcium sulfate were applied in powdered form and thoroughly mixed with the soil. Potassium was supplied as potassium chloride and phosphorus as mono-sodium phosphate except for applications above 500 pounds P_2O_5 per acre in which case the additional phosphorus was supplied as phosphoric acid. Potassium and phosphorus were usually applied in solution form and the soil thoroughly moistened to insure even distribution of the fertilizer. The soil was allowed to dry to optimum moisture content before planting. Soils were maintained at optimum moisture condition by supplying distilled water as needed during the growing period.

Results and Discussion

Exchangeable and water-soluble manganese concentrations of 25 naturally acid soils.

The objective of this study was to determine the concentrations of exchangeable and water-soluble manganese that might be found in naturally acid soils. Fifty-gram samples of 25 acid soils were placed in 50-ml, unstoppered Erlenmeyer flasks, brought to optimum moisture and allowed to incubate at greenhouse temperatures for two weeks. Water was added at intervals to maintain optimum moisture conditions.

At the end of the incubation period, the water-soluble and exchangeable manganese contents of the soils were determined essentially by the method of Sherman, McHargue, and Hodgkiss (49).

Data from this experiment are given in Table 12. The soils were divided into three groups according to their pH values to determine if any relationship existed between soil acidity, and exchangeable and water-soluble manganese concentrations of the soils. It is evident that little or no relation exists between pH and exchangeable manganese. However, the average water-soluble manganese concentration of 2.1 p.p.m. in the most acid soils was much higher than the average of 1.0 p.p.m. manganese for the intermediate acidity group or the average of 0.5 p.p.m. manganese for the least acid group.

Table 12. The pH values, exchangeable and water-soluble manganese concentrations of 25 naturally acid soils

Soil no.	Soil type	pH group	pH[1]	Exchange-able manganese	Water-soluble manganese (soil basis)
				ppm	ppm
1	Woodbridge silt loam		4.62	22.8	1.9
2	St. John sand	Below	4.68	1.2	0.0
3	Thurman sand		4.74	17.7	1.7
4	Fayette silt loam	pH 5.20	4.81	277.0	4.1
5	Carrington silt loam		5.05	109.2	6.3
6	Lindley sandy loam		5.11	34.7	0.3
7	Grundy silt loam		5.12	180.3	1.6
8	Carrington silt loam		5.20	165.8	0.6
		Average		101.1	2.1
9	Carrington silt loam		5.22	77.5	0.4
10	Edina silt loam		5.22	51.2	0.6
11	Carrington silt loam	Between	5.28	139.9	1.0
12	Muscatine silt loam	pH 5.21	5.28	178.5	1.5
13	Edina silt loam	and	5.30	13.1	0.4
14	Tama silt loam	5.40	5.30	203.3	2.0
15	Haig silt loam		5.32	85.0	0.2
16	Carrington silt loam		5.34	13.0	0.0
17	Carrington silt loam		5.34	18.2	0.7
18	Weller silt loam		5.38	638.4	2.7
		Average		141.8	1.0
19	Gosport si.clay loam		5.42	112.1	0.4
20	Carrington silt loam		5.50	8.2	0.0
21	Clinton silt loam		5.50	27.5	0.9
22	Weller silt loam	Above	5.50	403.0	0.4
23	Cecil sandy loam	pH 5.40	5.75	31.5	0.4
24	Marion silt loam		5.92	163.9	1.1
25	Shelby silt loam		6.10	44.5	0.4
		Average		113.0	0.5

[1]Determinations made with Beckman pH meter using 1:2.5 soil-water ratio.

Soils containing appreciable quantities of water-soluble manganese usually contained relatively large amounts of exchangeable manganese. Of the eleven soils containing more than 100 p.p.m. exchangeable manganese all but three contained more than 1.0 p.p.m. water-soluble manganese, and two of the three soils had pH values above 5.4. Two soils with less than 25 p.p.m. exchangeable manganese had high water-soluble manganese but both of these soils were strongly acid (No. 1 and 3). The two soils with the highest amounts of water-soluble manganese (No. 4 and 5) contained large amounts of exchangeable manganese and were also strongly acid.

From this experiment it is apparent that manganese toxicity might occur on soils of moderate acidity if the exchangeable manganese content is high. This is in agreement with the results of Hale and Heintze(18) who have reported manganese toxicity of crops growing on soils of moderate acidity (pH 5.6) which contained only 113 p.p.m. exchangeable manganese. Unfortunately no water-soluble manganese data were reported by these investigators.

Ten of the 25 soils contained 1.0 or more p.p.m. water-soluble manganese, a concentration which was found to be toxic to the growth of lespedeza in culture solutions in previous experiments. As soluble manganese was determined in a water extract in this experiment and reported on an oven-dry basis, it appeared likely that the manganese concentration of the soil solution might be considerably higher.

In order to test the validity of the method used in determining water-soluble manganese in this investigation, a study was made of the relation between the concentration of manganese in the soil solution of several soils and the concentrations of manganese in soil extracts obtained at two different soil-water ratios.

The soil solutions were displaced at optimum moisture conditions to prevent any increase in water-soluble manganese from waterlogging. The method described by Kelly (29) was used in displacing the soil solutions. Soils were only moderately tamped in the displacement cylinders so as to allow rapid displacement of the soil solution; in all cases the soil solutions were displaced in less than two hours.

Data from this experiment are given in Table 13. Approximately equal amounts of soluble manganese were obtained with 1:2 and 1:5 soil-water extracts. The soil solutions of the untreated soils contained as much manganese (soil basis) as either the 1:2 or 1:5 soil-water extract. However, the displaced solutions of the soils treated with calcium sulfate contained only about half the concentration of manganese obtained from the water extracts, when expressed on the soil basis. This indicates that the high concentration of water-soluble calcium in the soil solution may have suppressed the solubility of the exchangeable

Table 13. Water-soluble manganese concentrations of soils at varying moisture contents

Soil	Soil[1] Treatment	Soil pH	Water-soluble manganese: Soil solution	Soil solution[2]	1:2 soil-water extract[2]	1:5 soil-water extract[2]	Exchangeable[3] manganese
			ppm	ppm	ppm	ppm	ppm
Cecil sandy loam (Georgia)	check	5.18	13.2	1.3	0.4	0.6	5.6
	$CaSO_4$	4.58	70.7	6.1	10.7	11.8	5.1
	$CaSO_4$+P	4.55	56.7	5.3	11.5	8.8	6.6
Thurman sand (Iowa)	check	4.78	50.1	3.2	2.6	2.1	10.0
	$CaSO_4$	4.50	77.9	5.6	10.6	11.8	5.1
Carrington silt loam (Iowa)	check	4.87	3.2	0.8	0.8	1.1	7.5
	$CaSO_4$	4.58	15.7	4.3	10.3	14.4	20.2

[1] $CaSO_4$ applications = 2 m.e. Ca/100 gms. soil on Cecil and Thurman soils; 4 m.e. Ca/100 gms. soil on Carrington. P = 1000 lbs. P_2O_5 per acre (2,000,000 lbs.).

[2] Expressed on oven-dry soil basis.

[3] Water-soluble manganese not included.

manganese. The heavy application of phosphorus had no noticeable effect on the water-soluble manganese of the Cecil soil at any soil-water ratio.

In view of the results obtained in this experiment it is evident that in interpreting the data on soil extracts shown in the last column of Table 12 in terms of concentration of manganese in the soil solution, the values should be multiplied from four to ten fold, depending on the moisture content of the soils at time of displacement. The manganese concentrations thus obtained are, in most cases, within the range at which manganese was toxic to plants in culture solutions. These data were of considerable value in interpreting the results of subsequent experiments on the relation between the concentration of soluble manganese in soil extracts and plant growth.

Carrington silt loam

Experiment 1. The primary objective of this experiment was to study the relative effects of equivalent amounts of calcium carbonate and calcium sulfate on the soluble manganese content of the soil and on the growth and manganese content of sweet clover, on a Carrington silt loam of pH 4.8. Calcium carbonate was expected to reduce the soluble manganese in the soil because of the decreased soil acidity; whereas, calcium sulfate was expected to increase the water-soluble manganese with no increase in pH (11).

Treatments consisting of large applications of phosphate were also included as a means of eliminating soluble iron, aluminum, and possibly manganese as injurious factors without changing the H-ion concentration. Both strains of lespedeza were also grown in a less extensive study with only the low level of phosphorus. The crops were planted August 3, and harvested September 29, 1946. Sweet clover treatments were replicated three times and lespedeza treatments twice. Aluminum was determined colorimetrically by the "aluminon" method as given by

Peech and English (42). Other analytical methods were identical with those used in previous investigations.

Yields of sweet clover, pH values of the soil, and toxicity symptoms of plants from the various soil treatments are given in Table 14. Marked increases in growth were obtained from either calcium carbonate or the high phosphate treatments.

Table 14. The effect of various soil treatments on the yield of sweet clover grown on an acid Carrington silt loam, Experiment 1

Number	Treatment[1]	pH of soil	Average yield swt. clover per pot[2] (oven-dry)	Plant symptoms
			gms.	
1	check	4.81	1.00	Chlorotic leaf margin
2	1/4$CaSO_4$	4.71	1.03	" " "
3	1/2$CaSO_4$	4.72	0.77	" " "
4	$CaSO_4$	4.52	0.78	" " "
5	1/4$CaCO_3$	5.25	1.45	None
6	1/4$CaCO_3$+1/4$CaSO_4$	5.15	1.44	"
7	1/4$CaCO_3$+3/4$CaSO_4$	5.12	1.54	"
8	1/2$CaCO_3$	5.58	1.50	"
9	1/2$CaCO_3$+1/2$CaSO_4$	5.51	1.35	"
10	$CaCO_3$	6.28	1.52	"
11	P	4.74	1.32	Chlorotic leaf margin
12	P+1/4$CaSO_4$	4.83	1.16	" " "
13	P+1/2$CaSO_4$	4.59	1.37	" " "
14	P+$CaSO_4$	4.70	1.44	" " "
15	P+1/4$CaCO_3$	5.40	1.95	None
16	P+1/4$CaCO_3$+1/4$CaSO_4$	5.28	1.85	"
17	P+1/4$CaCO_3$+3/4$CaSO_4$	5.15	2.33	"
18	P+1/2$CaCO_3$	5.92	2.72	"
19	P+1/2$CaCO_3$+1/2$CaSO_4$	5.52	2.17	"
20	P+$CaCO_3$	6.30	1.98	"
21	3P	4.94	1.80	Chlorotic leaf margin
22	3P+1/4$CaSO_4$	4.80	1.25	" " "
23	3P+1/2$CaSO_4$	4.70	1.06	" " "
24	3P+$CaSO_4$	4.81	2.07	" " "

[1]Basic treatment = 50 lbs. K_2O, 50 lbs. P_2O_5 per acre; $CaSO_4$ and $CaCO_3$ = 10 m.e. Ca/100 gms. soil, P = 500 lbs. P_2O_5 per acre.

[2]Average of 3 replications.

L.S.D. =0.76 gms.

No additional benefit was obtained from applications of calcium carbonate greater than 2.5 m.m. of calcium. Calcium sulfate alone decreased yields in some cases and manganese toxicity symptoms were apparently more severe on plants from these treatments than plants from the check plots. Toxicity symptoms were evident on plants from the high phosphorus treatments not receiving applications of calcium carbonate (Treatments 11-14 and 21-24) despite the better growth. Symptoms consisted of a distinct yellowing and crimping of the leaf margin. The symptoms were not due to the high sulfate ion concentration of the soil as no symptoms were observed in treatments receiving large amounts of calcium sulfate plus calcium carbonate.

Chemical composition of plants from selected treatments are given in Table 15. The calcium content of the plants was not affected by applications of calcium carbonate or calcium sulfate. The phosphorus contents of plants from Treatments (1-10) were very low indicating that phosphorus was probably deficient in this soil. The iron content of the plants did not differ greatly except for Treatment 1, which was evidently contaminated.

Aluminum analyses of plants from selected treatments were made to determine if soluble aluminum might be an injurious factor in this soil. The heavy phosphate applications were found to be more effective than calcium carbonate in reducing the aluminum content of sweet clover. As toxicity symptoms of the plants were not alleviated by the heavy phosphate and no soluble aluminum was found in any of the soils, it is apparent that reduced plant growth on this soil was not due to aluminum toxicity.

Table 15. The effect of various soil treatments on the chemical composition of sweet clover grown on an acid Carrington silt loam, Experiment 1.

Number	Treatment[1]	Plant composition				
		Ca	P	Mn	Fe	Al
		%	%	ppm	ppm	ppm
1	Check	2.93	0.16	502	563	30
2	1/4$CaSO_4$	2.62	0.19	586	141	--
3	1/2$CaSO_4$	2.75	0.17	704	127	--
4	$CaSO_4$	2.71	0.17	532	171	35
5	1/4$CaCO_3$	2.41	0.13	37	141	--
8	1/2$CaCO_3$	2.42	0.14	33	130	--
10	$CaCO_3$	2.67	0.13	41	123	25
11	P	2.55	0.19	253	180	22
12	P+1/4$CaSO_4$	2.70	0.19	496	162	--
13	P+1/2$CaSO_4$	2.56	0.17	445	131	--
14	P+$CaSO_4$	2.52	0.20	381	165	15
15	P+1/4$CaCO_3$	2.15	0.21	90	112	--
20	P+$CaCO_3$	2.65	0.20	29	152	--
21	3P	2.21	0.30	436	136	--
22	3P+1/4 $CaSO_4$	2.70	0.30	848	144	--
23	3P+1/2$CaSO_4$	2.93	0.30	858	133	--
24	3P+$CaSO_4$	2.30	0.28	381	138	--

[1]Basic treatment = 50 lbs. K_2O, 50 lbs. P_2O_5 per acre; $CaSO_4$ and $CaCO_3$ = 10 m.e. Ca/100 gms. soil; P = 500 lbs. P_2O_5 per acre.

The manganese content of the plants was greatly affected by the treatments. A highly significant negative correlation was found between the yield of sweet clover and the manganese content of the plants at all three phosphorus levels. Plants receiving applications of calcium carbonate, regardless of the amount, contained very low concentrations of manganese. The calcium sulfate treated plants were usually higher in manganese content than the check plants. There was some indication that the manganese content of the plants in the highest calcium sulfate treatments was significantly lower than that of plants receiving smaller amounts of calcium sulfate. Phosphorus appeared to be slightly effective in lowering the manganese content of plants receiving intermediate amounts of phosphorus (Treatments 11-14) but no effect was evident at the high phosphorus level (Treatments 21-24).

The water-soluble manganese and calcium contents of soils from the majority of the treatments are given in Table 16. No water-soluble iron or aluminum was found in any of the soils. Approximately 75 per cent of the calcium added as calcium sulfate remained in a water-soluble form while the water-soluble calcium content of the soils receiving calcium carbonate was approximately equal to the check soils. It is evident that calcium carbonate is absorbed on the exchange positions while the calcium from calcium sulfate remains largely in a water-soluble form and is not absorbed appreciably on the exchange complex at these pH values.

No water-soluble manganese was found in soils receiving calcium carbonate. Soils receiving applications of calcium sulfate were much higher in water-soluble manganese than the check soils. Plants grown on soils containing relatively high concentrations of water-soluble manganese showed manganese toxicity symptoms and contained high amounts of manganese.

Table 16. The effects of various soil treatments on water-soluble[1] manganese and calcium contents of an acid Carrington silt loam, Experiment 1.

Number	Treatment[2]	Soil pH	Water-soluble Mn[3]	Water-soluble Ca
			ppm	m.e./100 gms.
1	Check	4.81	2.5	1.24
2	$1/4CaSO_4$	4.71	13.8	2.51
3	$1/2CaSO_4$	4.72	19.6	4.42
4	$CaSO_4$	4.52	5.4	8.47
10	$CaCO_3$	6.28	none	0.91
11	P	4.74	0.8	0.64
12	$P+1/4CaSO_4$	4.83	7.3	2.38
13	$P+1/2CaSO_4$	4.59	12.4	4.12
14	$P+CaSO_4$	4.70	4.1	8.31
21	3P	4.94	0.9	0.45
22	$3P+1/4CaSO_4$	4.80	8.9	2.43
23	$3P+1/2CaSO_4$	4.70	5.8	3.91
24	$3P+CaSO_4$	4.81	4.3	8.78

[1]One to five soil-water extract.
[2]Basic treatment = 50 lbs. K_2O and 50 lbs. P_2O_5 per acre; $CaSO_4$ and $CaCO_3$ = 10 m.e. Ca/100 gms. soil; P = 500 lbs. P_2O_5 per acre.
[3]Expressed on soil basis.

Treatments and yields of the lespedeza experiment on this soil are given in Table 17. The calcium sulfate treatment caused a definite decrease in yield of both strains of lespedeza, especially of strain L39. In contrast, strain L39 was benefited by calcium carbonate while L6 was not. Chemical composition data given in Table 18 show that strain L39 contained higher

Table 17. The effects of calcium sulfate and calcium carbonate applications on the yield of lespedeza grown on an acid Carrington silt loam, Experiment 1.

Number	Treatment[1]	pH	Average yield of lespedeza[2] (oven-dry basis) Strain L6	Strain L39	Both strains
			gms.	gms.	gms.
25	Check	5.00	3.42	2.16	2.79
26	$CaSO_4$	4.60	1.56	0.56	1.06
27	$CaCO_3$	6.15	3.03	2.63	2.83
28	$1/2CaCO_3+1/2CaSO_4$	5.45	3.14	3.05	3.10

L.S.D. for average of 2 strains =0.72

[1] $CaSO_4$ and $CaCO_3$ = 10 m.e. Ca/100 gms. soil.
[2] Average of 2 replications.

Table 18. The effects of calcium sulfate and calcium carbonate on the chemical composition of lespedeza grown on an acid Carrington silt loam, Experiment 1.

No.	Treatment[1]	Plant composition Ca Strain L6	Ca Strain L39	P Strain L6	P Strain L39	Mn Strain L6	Mn Strain L39	Fe Strain L6	Fe Strain L39	Al Strain L6	Al Strain L39
		%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm
25	check	1.86	2.27	0.22	0.26	275	356	166	172	45	-
26	$CaSO_4$	1.98	2.60	0.23	0.28	783	698	173	241	50	49
27	$CaCO_3$	1.91	2.47	0.19	0.22	29	43	153	180	49	-
28	$1/2CaCO_3+1/2CaSO_4$	1.79	2.30	0.19	0.21	20	24	149	158	39	--

[1] $CaSO_4$ and $CaCO_3$ = 10 m.e. Ca/100 gms. soil.

percentages of calcium, phosphorus, and iron than Strain L6 when grown under the same conditions. The higher calcium content of strain L39 may explain why it responded to calcium carbonate while strain L6 did not.

Plants in the calcium sulfate treatment were very chlorotic during early growth but had recovered to a great extent by harvest and the toxicity symptoms consisted of a considerable number of small, brown spots concentrated along the main veins of the older leaves. These symptoms were identical with those observed when lespedeza was grown in toxic concentrations of manganese in culture solutions in previous experiments.

The increased water-soluble manganese content of the soil, as well as the much higher manganese content and more severe manganese toxicity symptoms of the plants resulting from the calcium sulfate treatments, show clearly that the reduction in growth from calcium sulfate was due to manganese toxicity.

The greater tolerance of strain L6 to manganese toxicity as compared to strain L39 was again shown by the yields obtained with the calcium sulfate treatments. The yield of strain L6 on the calcium sulfate treated soil was 46 per cent of that of the check while that of strain L39 was only 26 per cent. The relatively high manganese content of strain L39 on the untreated soil indicates that the increased yield of this strain obtained from the calcium carbonate treatment was probably due largely to the reduction of manganese in the plant.

<u>Experiment 2</u>. The sweet clover experiment was planned primarily to confirm results obtained in the first experiment.

The principal purpose of the lespedeza study in this experiment was to determine the effects of different rates of calcium sulfate and of large amounts of phosphorus on the growth of lesoedeza. Sweet clover treatments were replicated four times and lespedeza treatments three times.

The treatments used in the sweet clover experiment on this soil are given in Table 19. This soil was less acid (pH 5.16) than the one used in the previous experiment (pH 4.81) and the effect obtained from either calcium carbonate or calcium sulfate was much less marked.

Calcium sulfate decreased the yield of sweet clover slightly as also shown in Table 19. A moderate application of manganese (Treatment 16) decreased the yield slightly more than calcium sulfate and manganese toxicity symptoms were more severe. When calcium carbonate was supplied with the manganese, no effect was apparent (Treatment 17). Toxicity symptoms of the plants grown on soils receiving calcium sulfate consisted of yellowish-white spots distributed along the leaf margin rather than the continuous chlorotic leaf margin exhibited in the more severe cases of manganese toxicity.

The heavy phosphorus applications, with or without calcium carbonate or calcium sulfate, approximately doubled the yield of sweet clover. Mono-calcium phosphate (Treatment 14) was not more effective than mono-sodium phosphate (Treatment 7).

Table 19. The effects of various soil treatments on the yield of sweet clover grown on acid Carrington silt loam, Experiment 2.

No.	Treatment[1]	pH	Average yield of sweet clover per pot[4] (oven-dry basis): Absolute	Relative
			gms.	%
1	check	5.16	3.72	100
2	$1/2CaSO_4$	4.87	3.27	88
3	$CaSO_4$	4.78	3.49	94
4	$1/2CaCO_3$	6.16	4.14	111
5	$CaCO_3$	7.34	4.19	113
6	$1/2CaCO_3+1/2CaSO_4$	5.94	4.59	123
7	P	5.55	7.62	205
8	$1/2CaSO_4$+P	4.88	6.95	187
9	$CaSO_4$+P	4.82	7.62	205
10	$1/2CaCO_3$+P	6.27	7.75	208
11	$CaCO_3$+P	6.98	7.51	202
12	$1/2CaCO_3+1/2CaSO_4$+P	5.77	7.95	214
13	$1/2MgCO_3$	6.23	4.32	116
14	P[2]	5.34	7.81	210
15	$CaSO_4$[3]+P	4.87	7.93	213
16	Mn	5.11	3.03	81
17	Mn+$CaCO_3$	6.37	4.19	113
				L.S.D. = 25

[1]Basic treatment = 100 lbs. K_2O and 100 lbs. P_2O_5 per acre. P = 1000 lbs. P_2O_5 per acre as NaH_2PO_4. $CaSO_4$ and $CaCO_3$ = 10 m.e. Ca/100 gms. of soil; $MgCO_3$ = 10 m.e. Mg/100 gms. of soil; Mn = 50 lbs. Mn as manganese chloride per acre.
[2]P added as mono-calcium phosphate.
[3]Commercial gypsum.
[4]Average of 4 replications.

Calcium carbonate was slightly beneficial with the low phosphorus treatments but no effect was obtained with the high phosphate level. Application of magnesium carbonate (Treatment 13) was also slightly beneficial.

The results from the lespedeza experiment on this soil (Table 20) displayed the same trends as those from the sweet clover experiment. All calcium sulfate treatments reduced the

Table 20. The effects of various soil treatments on the yield of lespedeza grown on an acid Carrington silt loam, Experiment 2.

Number	Treatment[1]	pH	Average yield of lespedeza per pot[2] (oven-dry basis) Absolute	Relative
			gms.	%
1	none	4.92	2.56	74
2	PK	5.12	3.47	100
3	$PK+1/4CaSO_4$	4.85	3.12	90
4	$PK+1/2CaSO_4$	4.77	2.75	79
5	$PK+3/4CaSO_4$	4.70	2.84	82
6	$PK+CaSO_4$	4.68	2.69	78
7	PK+Mn	5.09	3.05	88
8	$PK+Mn+CaCO_3$	6.48	3.54	102
9	$PK+CaCO_3$	6.30	3.57	103
10	$P_{10}K$	5.30	5.28	152
11	$P_{10}K+CaSO_4$	4.68	4.79	138
12	$P_{10}K+CaCO_3$	6.45	4.68	135
13	$P_{10}K+Mn$	5.10	4.96	143
				L.S.D.=20

[1]P = 100 lbs. P_2O_5 per acre, K = 100 lbs. K_2O per acre; $CaSO_4$ and $CaCO_3$ = 10 m.e. Ca/100 gms. soil. Mn = 50 lbs. manganese as manganese chloride per acre.
[2]Average of 3 replications.

yield of lespedeza, the smaller applications (Treatment 3) apparently being not as harmful as the heavier applications (Treatments 4 and 5). Calcium carbonate applications did not affect the yield of lespedeza although the plants receiving calcium carbonate were the only ones which did not show the brown spotting, characteristic of manganese toxicity, observed in the first experiment. Heavy phosphate applications increased the yield of lespedeza to a marked extent but spotting on the plants receiving high phosphate, with the exception of Treatment 12, was more severe than on the corresponding lower phosphate treatments. No plant analyses were made in this experiment.

The results from both experiments show that the beneficial effect of calcium carbonate was evidently due to the marked reduction in the water-soluble manganese content of the soil, and a corresponding decrease in absorption of manganese by the plant. Likewise, the detrimental effect of calcium sulfate was apparently due to the increase in the water-soluble manganese concentration of the soil and subsequent augmentation of manganese absorption by the plant. The greatly increased yields of sweet clover and lespedeza obtained from heavy applications of phosphate were due to the additional phosphorus supplied, rather than to any alleviation of manganese toxicity.

The relative effects of calcium carbonate and calcium sulfate on the growth and manganese content of lespedeza and sweet clover

Ten acid soils, varying in pH values from 4.78 to 5.62, were used in this study. Lespedeza was grown on all ten soils and sweet clover on three of the soils. Soils with low exchange capacities (both Cecil soils and the Thurman sand) received 2 m.e. of calcium per 100 grams of soil while all other soils received twice as much calcium. The effect of large applications of phosphorus on the growth of sweet clover and lespedeza was also studied on two of the soils.

Table 21. The effects of $CaSO_4$ and $CaCO_3$ on growth and chemical composition of sweet clover, soil reaction, and water-soluble soil manganese

Treatment[1]	Soil: Water-soluble Mn[2]	Soil: pH	Plant composition: Mn	Plant composition: Ca	Plant composition: P	Yields (oven-dry basis): Absolute	Yields (oven-dry basis): Relative
	ppm		ppm	%	%	gms	%
			Soil - Gosport silt clay loam				
check	0.6	4.97	320	2.48	0.28	0.91	100
$CaSO_4$	9.8	4.76	724[+]	3.02	0.30	0.64	70
$CaCO_3$	none	5.73	71	2.92	0.29	1.27	140
			Soil - Weller silt loam				
check	none	5.42	255	3.96	0.56	0.72	100
$CaSO_4$	3.3	5.04	600[+]	4.32	0.34	0.53	74
$CaCO_3$	none	6.22	83	4.02	0.39	0.72	100
P	none	5.42	223	3.47	1.07	1.29	179
P+$CaSO_4$	2.5	5.05	427[+]	3.71	0.84	1.04	144
P+$CaCO_3$	none	6.32	93	3.59	0.80	1.50	208
			Soil - Fayette silt loam				
check	none	5.55	134	2.29	0.30	1.99	100
$CaSO_4$	0.2	5.30	152	2.46	0.26	2.18	110
$CaCO_3$	none	6.58	51	2.70	0.30	2.29	115

[1] $CaSO_4$ and $CaCO_3$ = 4 m.e. Ca/100 gms. soil.
P = 1000 lbs. P_2O_5 per acre.
[2] Expressed on soil basis, 1:2 soil-water extract.
[3] Average of 3 replications.
[+] Marked manganese toxicity symptoms of foliage.

Yields and manganese and calcium contents of sweet clover grown on three acid soils are given in Table 21. Calcium sulfate decreased the yield of sweet clover on the Gosport and Weller soils but had no effect on the Fayette soil. Sweet clover was also planted on the Cecil (Ga.) soil but plants not receiving calcium carbonate grew so poorly that no yield records or analyses were made. Manganese toxicity symptoms were evident on the plants grown on the Gosport, Weller and Cecil soils as shown in Fig. 12. Where these symptoms occurred a high manganese content was found in the plants and in the soil extract. Calcium carbonate applications were beneficial on the Gosport soil but no significant increases from calcium carbonate were obtained on the Weller or Fayette soils.

The high phosphate treatment on the Weller soil resulted in greatly increased growth of sweet clover. The phosphorus content of the plants grown with high phosphorus on this soil was unusually high, exceeding one per cent when no calcium treatment was added. No marked decreases in the manganese content of the plants or of the soil extracts were brought about by the large application of phosphate.

Data from the lespedeza experiment are given in Table 22. Applications of calcium sulfate reduced yields of lespedeza on six of the ten soils included in the experiment. In no case were yields of lespedeza increased significantly by calcium sulfate. Applications of calcium carbonate were beneficial on the Thurman and Cecil soils, the two soils on which the plants showed manganese toxicity symptoms and made poor growth without calcium carbonate additions. On the other soils, which contained relatively large amounts of exchangeable calcium, the calcium carbonate treatment was ineffective and in two cases (Edina and Tama) reduced yields significantly. The effects of

Table 22. The effects of $CaSO_4$ and $CaCO_3$ on growth and chemical composition of lespedeza, soil reaction, and water-soluble soil manganese

(Strain L6 Korean lespedeza)

Treatment[1]	Soil: Water-soluble Mn[2]	Soil: pH	Plant composition: Mn	Plant composition: Ca	Plant composition: P	Yields[3] (oven-dry basis): Absolute	Yields[3] (oven-dry basis): Relative
	ppm		ppm	%	%	gms.	%
		Soil - Cecil sandy loam (N.C.)					
check	none	5.55	255	1.54	-	1.07	100
$CaSO_4$	1.3	4.97	697+	1.89	-	0.65	61
$CaCO_3$	none	6.01	99	1.52	-	1.15	107
		Soil - Gosport silt clay loam (July, August)					
check	0.3	5.11	310	1.34	-	1.05	100
$CaSO_4$	10.1	4.85	892+	1.38	-	0.55	52
$CaCO_3$	none	5.58	82	1.66	-	1.05	100
		Soil - Thurman sand					
check	2.6	4.78	2066+	0.85	0.28	0.23	100
$CaSO_4$	10.6	4.50	3296+	1.52	0.20	0.045	22
$MgCO_3$	none	5.99	359	0.49	0.35	1.36	591
$CaCO_3$	none	5.75	190	1.40	0.37	1.39	604
		Soil - Gosport silt clay loam					
check	0.6	4.97	292	1.43	0.32	0.96	100
$CaSO_4$	9.8	4.76	1049+	1.85	0.30	0.57	59
$CaCO_3$	none	5.73	115	1.78	0.29	0.75	78
		Soil - Gosport silt clay loam - Strain L39					
check	0.6	4.97	276	1.77	0.30	0.78	100
$CaSO_4$	9.8	4.76	1091+	2.36	0.37	0.46	59
$CaCO_3$	none	5.73	95	2.25	0.28	0.73	94
		Soil - Weller silt loam					
check	none	5.42	179	1.87	0.29	0.57	100
$CaSO_4$	3.3	5.04	272	2.06	0.29	0.55	96
$CaCO_3$	none	6.22	97	1.91	0.31	0.47	82
P	none	5.42	182	1.77	0.35	1.20	211
P+$CaSO_4$	2.5	5.05	289	1.93	0.38	1.21	212
P+$CaCO_3$	none	6.32	92	1.85	0.36	1.25	219

[1] $CaSO_4$ and $CaCO_3$ = 2 m.e. Ca/100 gms. of soil on Thurman and Cecil soils, 4 m.e. Ca/100 gms., on other soils, P = 1000 lbs. P_2O_5 per acre. Significant increase or decrease.

[2] Expressed on soil basis, 1:2 soil-water extract.

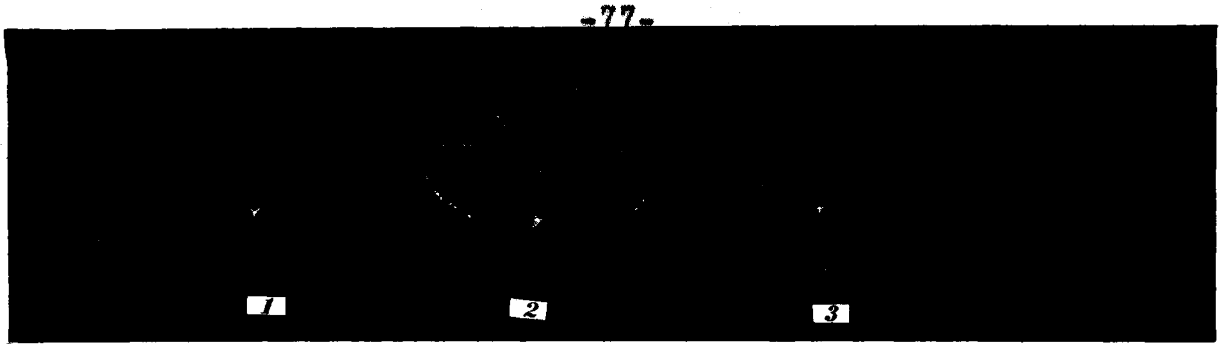
[3] Average of 3 replications.

+ Marked manganese toxicity symptoms of foliage.

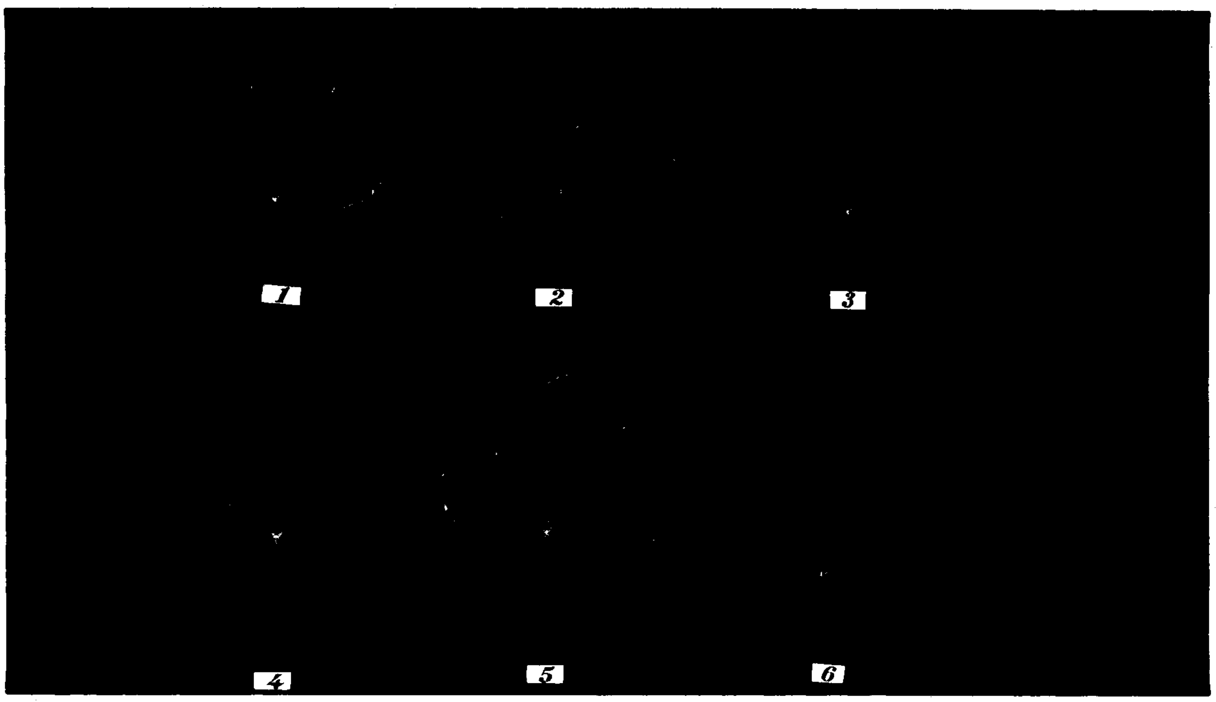
Table 22 (continued)

Treatment[1]	Soil: Water-soluble Mn^{2}	Soil: pH	Plant composition: Mn	Plant composition: Ca	Plant composition: P	Yield[3] (oven-dry basis): Absolute	Yield[3] (oven-dry basis): Relative
	ppm		ppm	%	%	gms.	%
Soil - Cecil sandy loam (Ga.)							
check	0.4	5.18	807+	0.71	0.14	0.16	100
$CaSO_4$	10.7	4.58	1356+	1.58	0.18	0.13	81
$CaCO_3$	none	5.95	141	1.25	0.18	0.26	163
P	0.4	5.62	1646+	0.58	0.56	0.38	238
$P+CaSO_4$	11.5	4.55	1773+	2.05	0.43	0.13	81
$P+CaCO_3$	none	6.17	286	1.69	0.48	0.75	469
Soil - Carrington silt loam (1)							
check	0.8	4.87	354	1.61	0.23	0.73	100
$CaSO_4$	10.3	4.58	760+	1.72	0.27	0.45	62
$CaCO_3$	none	5.51	82	1.70	0.23	0.59	81
Soil - Carrington silt loam (2)							
check	0.2	5.01	164	1.84	0.28	1.36	100
$CaSO_4$	1.6	4.78	322	2.03	0.34	1.11	81
$CaCO_3$	none	5.74	55	1.92	0.28	1.10	81
Soil - Fayette silt loam							
check	none	5.55	159	1.77	0.33	0.78	100
$CaSO_4$	0.2	5.30	201	1.66	0.35	0.87	112
$CaCO_3$	none	6.58	60	1.68	0.36	0.63	81
Soil - Tama silt loam							
check	none	5.52	61	1.11	0.18	0.83	100
$CaSO_4$	none	5.22	74	1.28	0.17	0.91	110
$CaCO_3$	none	6.22	30	1.30	0.17	0.65	78
Soil - Edina silt loam							
check	0.4	5.08	176	1.66	0.26	0.62	100
$CaSO_4$	1.0	4.71	267	1.86	0.26	0.38	61
$CaCO_3$	none	6.05	68	1.55	0.24	0.48	77

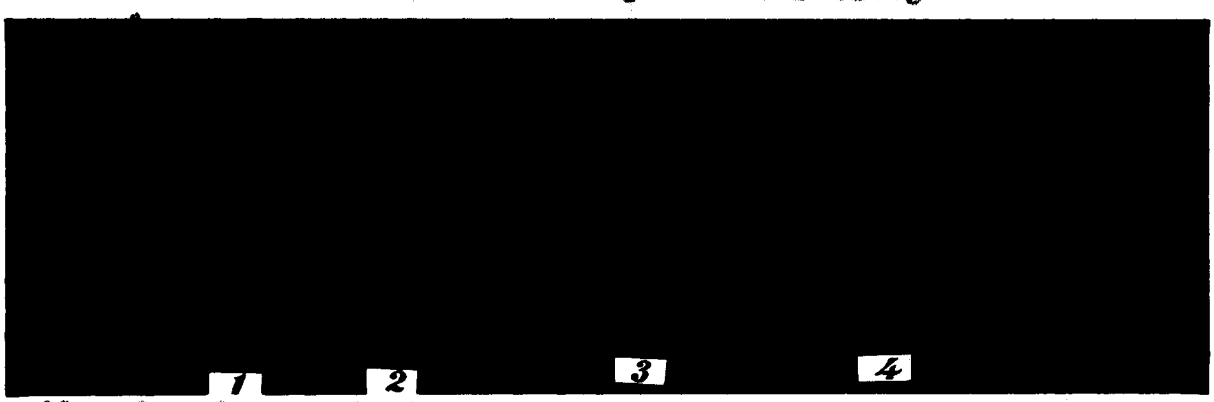
[1] $CaSO_4$ and $CaCO_3$ = 2 m.e. Ca/100 gms. of soil on Thurman and Cecil soils, 4 m.e. Ca/100 gms. on other soils. P = 1000 lbs. P_2O_5 per acre.
Significant increase or decrease.
[2] Expressed on soil basis, 1:2 soil-water extract.
[3] Average of 3 replications.
+Marked manganese toxicity symptoms of foliage.



Gosport si. clay loam   Ck. $CaSO_4$ $CaCO_3$



Weller silt loam   1. Ck.   2. $CaSO_4$ 3. $CaCO_3$
4. P   5. P+$CaSO_4$ 6. P+$CaCO_3$



Cecil s.l.   Ck. $CaSO_4$ $CaCO_3$ P+$CaSO_4$

Fig. 12. Manganese toxicity symptoms of sweet clover.

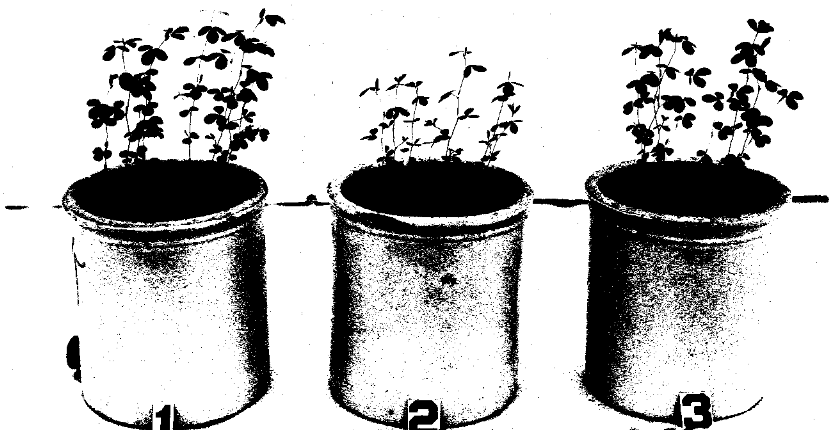
calcium carbonate and calcium sulfate on the growth of lespedeza on several soils are shown in Figs. 13-16.

The low calcium contents of the plants grown on the untreated Thurman and Cecil soils indicated that calcium deficiency was a possible limiting factor on these soils. As magnesium carbonate was as beneficial to plant growth on the Thurman sand as calcium carbonate it might be presumed that a deficiency of total bases, rather than calcium alone, contributed to the poor growth of plants on this soil.

Although calcium sulfate increased the calcium content of the plants grown on the Thurman and Cecil soils significantly there was a decrease in growth, indicating that soluble manganese was the primary factor responsible for poor plant growth on these soils.

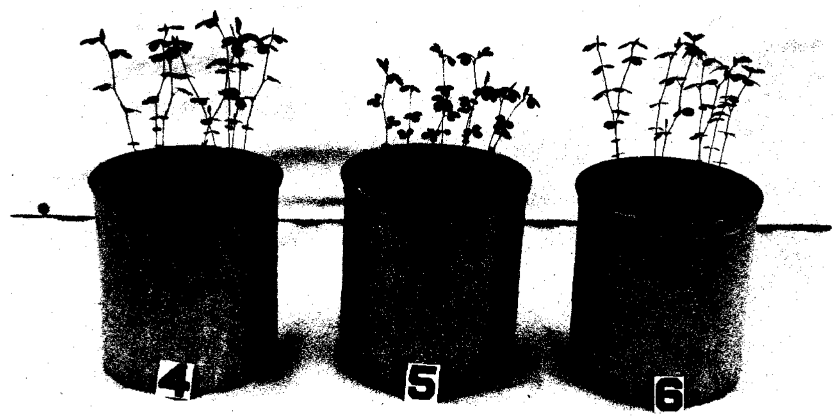
Two experiments were carried out on the Gosport soil to determine if climatic conditions might affect plant response to the different treatments. The first crop was grown during July and August and the second during September and October. The calcium sulfate treatment decreased the yield of lespedeza significantly in each experiment. Both strains of lespedeza, L6 and L39, were grown on this soil and were injured to the same extent by the calcium sulfate treatment.

The decrease in yield obtained with calcium carbonate on several of the soils was thought to be due to some disturbance



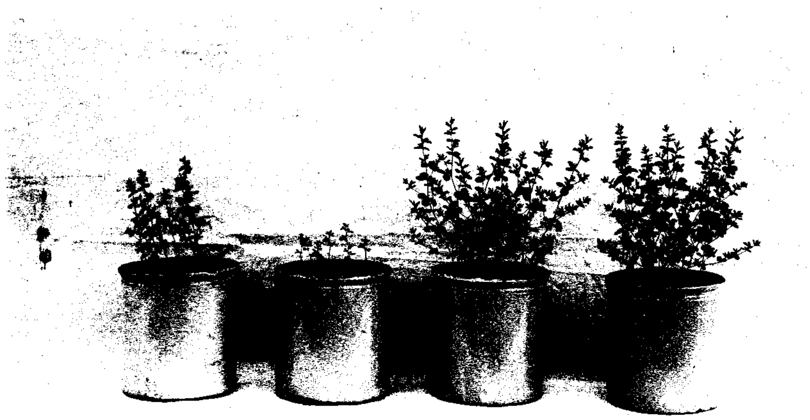
Ck. $CaSO_4$ $CaCO_3$

Cecil sandy loam (N. C.)



Ck. $CaSO_4$ $CaCO_3$

Gosport silty clay loam

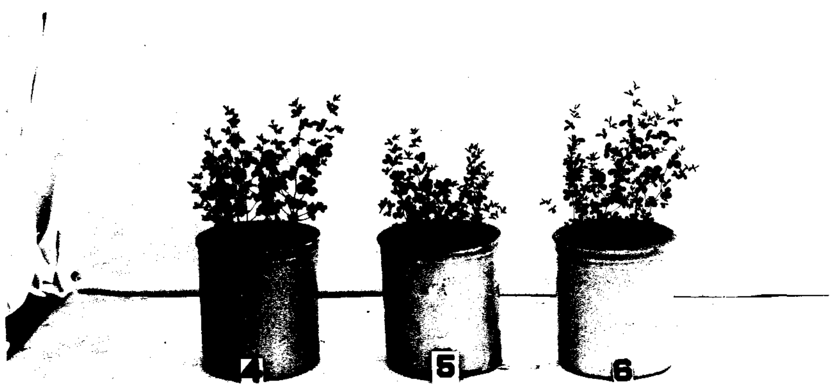


Ck. $CaSO_4$ $MgCO_3$ $CaCO_3$

Thurman sand

Fig. 13. Growth of lespedeza on Cecil, Gosport, and Thurman soils.

Ck. $CaSO_4$ $CaCO_3$
Tama silt loam



Ck. $CaSO_4$ $CaCO_3$
Carrington silt loam (1)

Ck. $CaSO_4$ $CaCO_3$
Carrington silt loam (2)

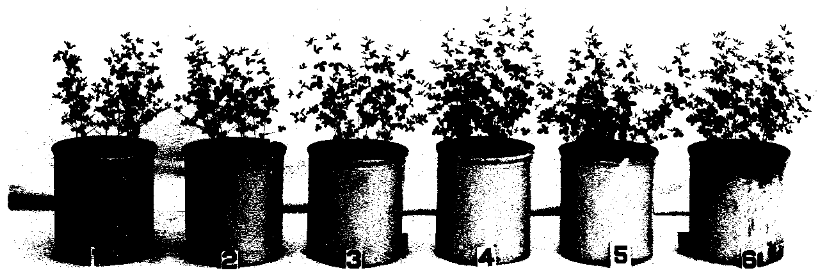
Fig. 14. Growth of lespedeza on Tama and Carrington soils.

Ck. $CaSO_4$ $CaCO_3$
Edina silt loam

Ck. $CaSO_4$ $CaCO_3$
Fayette silt loam

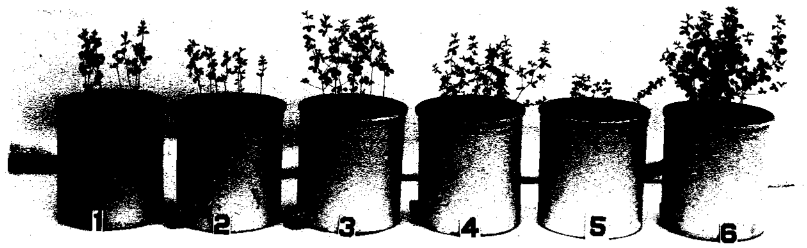
Ck. $CaSO_4$ $CaCO_3$ Ck. $CaSO_4$ $CaCO_3$
Strain L6 Strain L39
Gosport silty clay loam

Fig. 15. Growth of lespedeza on Edina, Fayette, and Gosport soils.



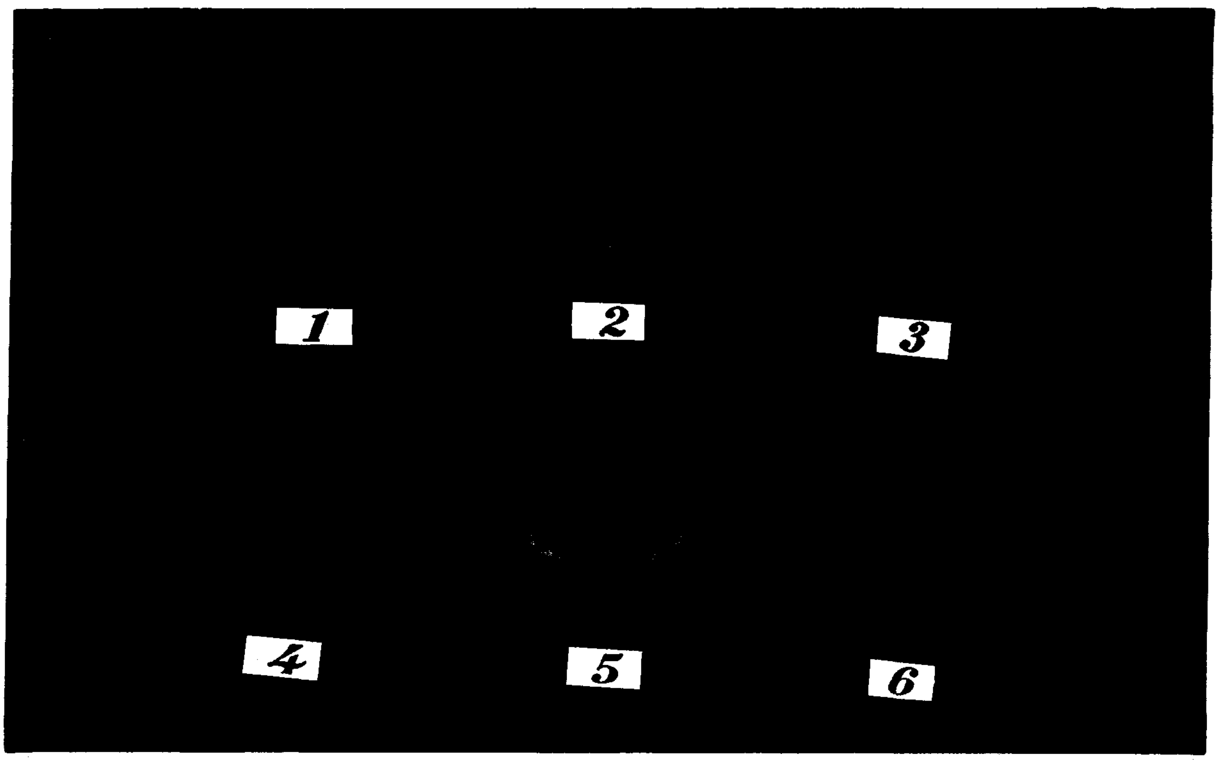
Ck. $CaSO_4$ $CaCO_3$ P P+$CaSO_4$ P+$CaCO_3$

Weller silt loam



Ck. $CaSO_4$ $CaCO_3$ P P+$CaSO_4$ P+$CaCO_3$

Cecil sandy loam (Ga.)



Cecil sandy loam (Ga.)

Fig. 16. Growth and manganese toxicity symptoms of lespedeza on Weller and Cecil soils.

in the phosphorus absorption and utilization in the plant but phosphorus analyses of the plants did not support this theory.

Calcium sulfate was evidently a satisfactory source of calcium as plants from this treatment usually contained more calcium than plants from either the check or calcium carbonate treatments. However, the outstanding effect of calcium sulfate on plant composition was the greatly increased manganese content. In all cases where the yield of lespedeza was decreased significantly by calcium sulfate an accompanying increase in the manganese content of the plant and water-soluble manganese concentration of the soil was obtained. A comparison of the toxicity symptoms of lespedeza growing in a culture solution containing a toxic concentration of manganese and toxicity symptoms of lespedeza grown on calcium sulfate treated soils are shown in Fig. 17.

Only comparatively low concentrations of manganese were found in the water extracts of the untreated Thurman and Cecil (Ga.) soils although the manganese content of the plants grown on these soils was high and manganese toxicity symptoms were severe. The high concentration of manganese in the untreated Cecil and Thurman soil solutions (Table 13) explain why plant growth was so poor and manganese toxicity symptoms so severe on these soils. Concentrations of manganese found in the displaced soil solutions were much higher than those causing extreme injury to lespedeza in culture solutions.

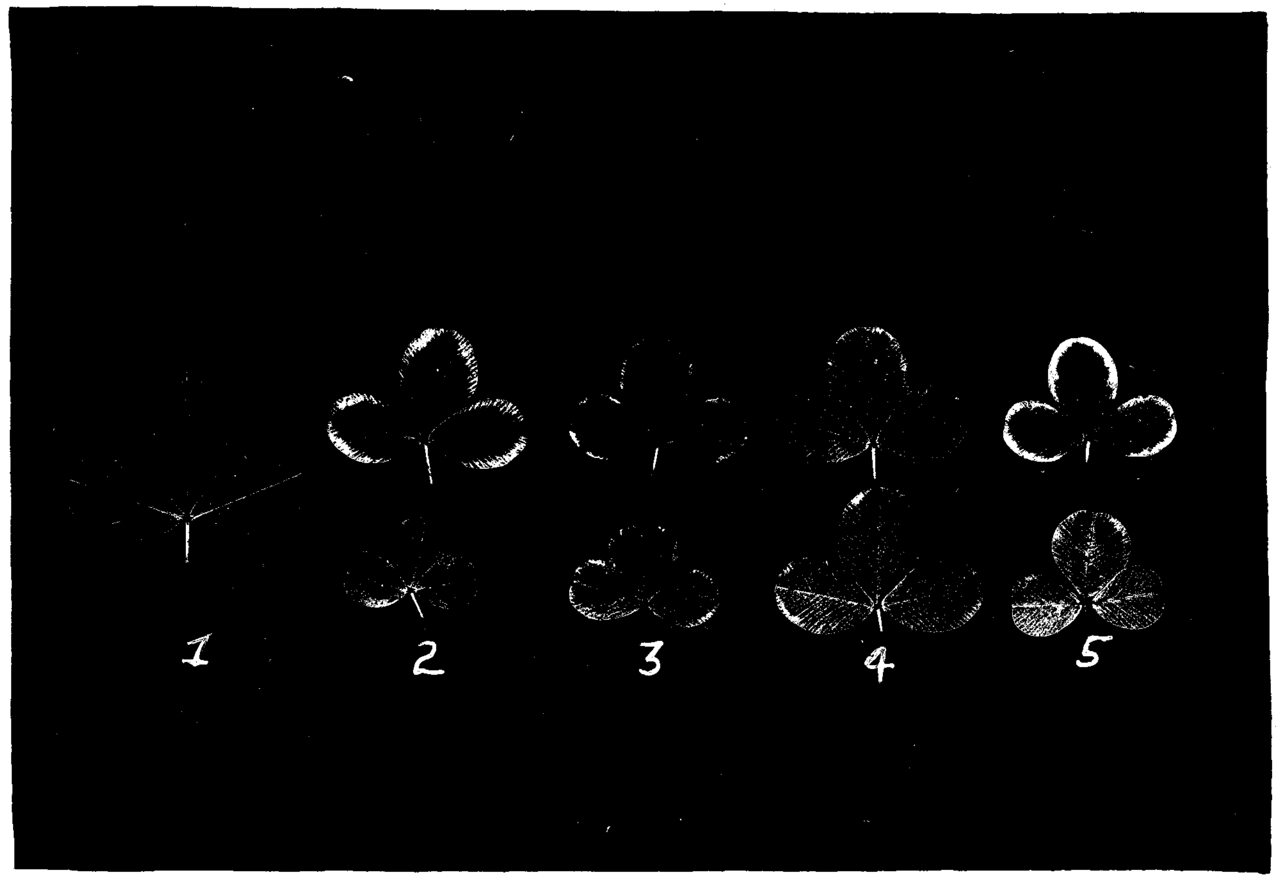


Fig. 17. Manganese toxicity symptoms of lespedeza grown in culture solution and in acid soils.

1. Cecil soil (limed)
2. Culture soln. (5ppm Mn)
3. Cecil soil ($CaSO_4$)
4. Cecil soil (untreated)
5. Gosport soil ($CaSO_4$)

The large application of phosphate on the Weller soil resulted in marked increases in yields, evidently due to the additional phosphorus supplied. Somewhat similar results were obtained on the Cecil (Ga.) soil except that the high phosphate in combination with calcium sulfate did not increase yields. The high manganese content of the plants and extreme toxicity symptoms (Fig. 16) indicate that manganese toxicity was responsible for the poor growth of lespedeza in this case.

Results from this experiment corroborate those of the Carrington experiments. Calcium carbonate was beneficial only on soils which contained toxic concentrations of soluble manganese. Calcium sulfate was not beneficial on any soil and was detrimental on soils where high concentrations of manganese were brought into solution. The increased yields resulting from large applications of phosphorus were due to the additional phosphorus supplied.

GENERAL DISCUSSION

All five legumes studied in the investigation were found to be injured by concentrations of soluble manganese lower than that occurring in certain acid soils. The minimum concentration of manganese necessary for injury to the various legumes varied considerably. The legumes ranked in order of their sensitivity to soluble manganese were: lespedeza, sweet clover, soybeans and cowpeas, peanuts. This ranking was verified when the legumes were grown on a strongly acid, high-manganese soil for observational study.

The tolerance of a legume to manganese toxicity is presumably due to two factors: The amount of manganese absorbed by the plant, and the ability of the plant to withstand high concentrations of manganese absorbed. Thus, the difference in tolerance displayed by peanuts and sweet clover, which absorbed approximately the same amounts of manganese, would be explained by the relatively greater ability of peanuts to endure high concentrations of manganese within the plant. On the other hand, the difference in tolerance displayed by sweet clover and lespedeza, both of which were injured by small quantities of manganese within the plant, would be due to the relatively greater absorption of manganese by lespedeza.

The relatively good growth of lespedeza on many acid soils is evidently not due to its tolerance to manganese toxicity. The 1.0 p.p.m. concentration of manganese necessary for injury to lespedeza was of the same magnitude as that reported by Olsen (39) as being toxic to the more sensitive plant species included in his investigation.

No evidence was found that calcium reduced manganese toxicity as reported by Hewitt (19). The conflicting results might be explained by the difference in crops used. The satisfactory growth of lespedeza at the low calcium level used in the culture solution experiments in this investigation (12 p.p.m.) indicate that the generally accepted classification of lespedeza as an acid tolerant legume might be largely due to its ability to grow well in soils where available calcium is low.

The negative effect of phosphorus on manganese toxicity found in the investigation is directly opposed to the conclusions of Kelley (27) and Bortner (5). An explanation of this differential response to phosphorus is apparent upon closer study of their data. Kelley (27) applied phosphate to soils high in soluble manganese and obtained increased yields but manganese toxicity symptoms of the plants were still evident indicating that the beneficial effect of the phosphate fertilizer was due to the additional phosphorus available or reduced toxicity of aluminum (43), rather than to any effect on manganese toxicity. Bortner (5) added phosphate to soil in the form of tri-sodium phosphate but made no determination of the change in soil reaction. Any benefit obtained under these circumstances could be due to the direct effect of additional phosphorus or to a decrease in soluble manganese by precipitation under the less acid soil conditions. Bortner also found that removal of phosphorus from a nutrient solution lowered the manganese concentration at which injury occurred. It is questionable, however, if poor growth and toxicity symptoms of plants grown in such a solution could be attributed to manganese toxicity alone.

The beneficial effect of iron in reducing the toxicity of manganese to lespedeza is in agreement with the results of several investigators (6, 20, 55) who worked with other crops. The optimum iron-manganese ratios of the culture solutions were 10:1 and 1:2 in the two experiments conducted; no evidence was found to support Somers and Shive's (54) theory that an iron-manganese ratio of approximately 2.0 is necessary for normal growth.

Results from the soil experiments indicate that soluble manganese is a primary factor affecting the growth of legumes on certain acid soils. This theory was supported by the definite toxicity symptoms of the plant foliage, high manganese content of the plant material, and the relatively high water-soluble manganese concentration in the soils.

Applications of calcium carbonate were beneficial to plant growth on acid soils containing high amounts of water-soluble manganese. This beneficial effect was due, at least in part, to the reduction of soluble manganese in the soil. Application of calcium sulfate to acid soils was detrimental because of the increased soluble manganese in the soil brought about by the treatment rather than to a failure to supply an available form of calcium. Calcium sulfate increased the soil acidity in all cases. The calcium content of plants receiving calcium sulfate were higher in most instances than those receiving calcium carbonate. No evidence was found that high concentrations of manganese depressed calcium absorption as reported by Fried and Peech (11).

The water-soluble manganese concentration of a soil is not affected to any extent by heavy applications of phosphate. On some soils manganese toxicity symptoms of plants were more severe where large amounts of phosphate were applied. In view of the information obtained from the iron-manganese culture solution experiments, it appears that the increased toxicity might be due to the decreased availability of iron, either in the plant or soil or a combination of the two.

SUMMARY

Results of investigations to determine the relative importance of soluble manganese as a toxic factor affecting the growth of various legumes in culture solutions and in acid soils, may be summarized as follows:

1. Concentrations of manganese varying from 1 to 10 p.p.m. were found to be injurious to the five legumes studied.

2. The order of the legumes according to their relative sensitivity to soluble manganese was: lespedeza, sweet clover, soybeans and cowpeas, and peanuts.

3. The legumes differed greatly in the amounts of manganese absorbed, cowpeas absorbing the largest amount of manganese and peanuts and sweet clover the least.

4. Manganese concentrations of leaves were much higher than those of stems.

5. Manganese toxicity symptoms of the various legumes differed greatly. Sweet clover and peanut symptoms consisted of chlorotic leaf margins and lespedeza symptoms, of chlorotic leaf margins and spotting of the leaves. Pale-green, irregular areas between the main veins characterized the toxicity symptoms of soybeans and the cowpea toxicity symptoms were minute purple-red spots distributed over the entire leaf.

6. Iron deficiency symptoms of soybeans were not identical

with manganese toxicity symptoms, as has been claimed by other investigators.

7. Two strains of Korean lespedeza differed significantly in their tolerance to manganese toxicity in culture solutions. The strain showing the least tolerance grew relatively poorer under acid soil conditions and relatively better under more alkaline soil conditions.

8. An increase in the calcium concentration of the culture solution from 12 to 60 or to 300 p.p.m. was ineffective in reducing manganese toxicity to lespedeza. There was some indication that manganese toxicity was increased at the higher calcium levels.

9. No alleviation of manganese toxicity was obtained by increasing the phosphorus in the culture solution from 2 to 20 p.p.m.

10. An increase in the concentration of iron in culture solutions up to 1 p.p.m. resulted in marked reduction of the toxicity from a given concentration of manganese. Addition of iron beyond 1 p.p.m. resulted in decreased growth regardless of the manganese concentration.

11. The iron-manganese ratio of the culture solution was not a primary factor in the growth of lespedeza. Good growth was obtained over a wide range of iron-manganese ratios.

12. The beneficial effect of iron in reducing manganese toxicity was due to a decrease in manganese absorbed by the plant rather than an increase in iron absorption.

13. Legumes making poor growth on certain acid soils exhibited toxicity symptoms identical with those observed on plants grown in culture solutions containing toxic concentrations of manganese.

14. Plants exhibiting the most severe manganese toxicity symptoms contained the highest amounts of manganese. When sweet clover or lespedeza contained above 400 p.p.m. manganese a reduction in yield, proportional to the excess manganese, was obtained.

15. The variation in water-soluble manganese in different soils is related to the pH and exchangeable manganese content of the soils.

16. Applications of calcium carbonate were beneficial to plant growth on acid soils containing high amounts of water soluble manganese. This beneficial effect was due, at least in part, to the reduction of soluble manganese in the soil brought about by the increase in pH.

17. Applications of calcium sulfate to acid soils were detrimental because of the increased soluble manganese in the soil and a corresponding increase in the manganese content of the plant brought about by the treatment rather than failure to supply an available form of calcium.

18. Calcium sulfate increased the soil acidity in all cases.

19. Heavy applications of phosphate fertilizer to acid soils had no effect on the water-soluble manganese content of the soils.

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